

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES**

2020

Bogotá, D.C.,

Abogada

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ASUNTO

Radicación	10 054 926
Trámite	11
Evento	378
Actuación	519
Oficio	ST - - 17 9 0 3
Folios	ST

Patente de Invención



Patente de Modelo de Utilidad

Via Nacional

Via PCT (Fase Nacional)



Capítulo I



Capítulo II

No. Solicitud Internacional

PCT/US2008/079042

Fecha de Presentación Internacional

07/Octubre/2008

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 48 a 88	07/Mayo/2010
Reivindicaciones:	Folios 89 a 93	07/Mayo/2010
Dibujos:	Folios 95 a 98	07/Mayo/2010
Prioridad:	US 60 978 303	08/Octubre/2007

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "Formas sólidas de ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico y métodos para su uso".

El problema planteado en esta solicitud consiste en la necesidad de suministrar formas cristalinas del compuesto ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico con mejores propiedades como solubilidad y estabilidad.

Para resolver este problema técnico la solicitud en estudio proporciona un compuesto cristalino, que es ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico o una sal del mismo.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07D237/34, A61K31/505, A61P1/00//25/00.

4. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de las reivindicaciones 28-31 no es patentable de acuerdo con el Art citado.

5. De la Solicitud de Patente

Titulo

El título contiene el término "métodos para su uso" que hace alusión a temas que son excluidos o exceptuados de patentabilidad. Se sugiere eliminar el término de tal manera que el título se ajuste a las categorías de producto.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicaciones 1 – 4, 7 – 9, 13 - 14, y 17 no son claras porque hacen referencia a una forma cristalina, pero ésta no están definidas por sus características técnicas esenciales como el patrón de difracción de rayos X en mono cristal, el patrón de difracción de rayos X en polvo (DRXP) con por lo menos los 20 picos correspondientes a las intensidades de los haces difractados o a los espacios interplanares d_{hkl} según los índices de Miller (hkl) medidos de acuerdo con el ángulo de difracción de Bragg 2θ en el intervalo más representativo comprendido entre 5° hasta 90° , las espectroscopías Raman e IR, la espectroscopia RMN- C^{13} , o, los métodos de análisis térmico: TGA, DTA y DSC. Se solicita caracterizar la forma cristalina de acuerdo a lo que se encuentre debidamente soportado en la descripción.

Las reivindicaciones 4, 5, 9, 10, 14 y 15 no son claras cuando emplean el término "aproximadamente", "y/o", "uno o más", los cuales son imprecisos, por lo cual no permiten distinguir la invención del estado de la técnica. Se sugiere sustituirlos por términos precisos o rangos concreto.

Las reivindicaciones 5, 10 y 13 no son claras porque hacen referencia a una forma cristalina caracterizada mediante difracción de rayos X en polvo, pero este no cuenta con por lo menos los 20 picos correspondientes a las intensidades de los haces difractados o a los espacios interplanares d_{hkl} según los índices de Miller (hkl) medidos de acuerdo con el ángulo de difracción de Bragg 2θ en el intervalo más representativo comprendido entre 5° hasta 90° . Se solicita caracterizar la forma cristalina con por lo menos los 20 picos mencionados, de acuerdo a lo que se encuentre debidamente soportado en la descripción.

Las reivindicaciones 6, 11 y 12 no son claras porque hacen referencia a los dibujos de acuerdo a la expresión, "como el que se muestra en la Figura...". Se sugiere eliminar la referencia a los dibujos.

La reivindicación 17 no es clara porque se refiere al compuesto maleato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato, pero está redactado como "(S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-maleato de trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato". Se sugiere hacer la corrección correspondiente.

Las reivindicaciones 17 y 20 no están soportados en la descripción porque en ninguna parte de la misma se encuentra que el compuesto maleato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propa-

noato haya sido obtenido o probado. Se sugiere eliminar estas reivindicaciones del pliego reivindicatorio.

6. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad 08 de octubre de 2007 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D1	WO2007/089335	Multicyclic amino acid derivatives and methods of their use	09/Agosto/2007
D2	Libro ¹	Handbook of pharmaceutical salts	2002
D3	Artículo ²	Crystalline solids	2001

D1
http://worldwide.espacenet.com/publicationDetails/originalDocument;jsessionid=575E1CD2973DE1A251B61F8188C28394.espacenet_levelx_prod_6?CC=WO&NR=2007089335A2&KC=A2&FT=D&ND=&date=20070809&DB=&locale=en_EP, divulga (pg. 92, compuesto 539) el compuesto ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico, y composiciones farmacéuticas que lo contienen.

D2 (fls 137 - 143) divulga ácidos y bases empleados en la formación de sales farmacéuticamente aceptables, así como el mejoramiento de las propiedades fisicoquímicas y mecánicas de los fármacos mediante la formación de sales.

D3 (folios 146 – 157) enseña que la formación de hidratos (pseudopolimorfos) es la formación de sólidos cristalinos en cuya estructura contienen moléculas de solvente (agua), que es un trabajo habitual en el campo de preformulación farmacéutico y que su caracterización se puede hacer mediante difracción de rayos X en polvo.

7. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

La reivindicación 1 consiste en un compuesto cristalino, que es ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico o una sal del mismo.

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1
Ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico o una sal del mismo.	Ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico (pg. 92, compuesto 539).

Las características esenciales del compuesto de la reivindicación 1 habían sido divulgadas previamente en D1, el cual da a conocer el compuesto ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico (pg. 92, compuesto 539). Si bien es cierto, no dice expresamente que es un compuesto cristalino, el solicitante tampoco proporciona las características técnicas esenciales del compuesto como una forma cristalina.

¹ Handbook of pharmaceutical salts. Properties, selection and use; Wiley-VCH; 2002; pgs. 19, 28, 51-66, 162-168, 292 y 309; fls 137-143.

² Vippagunta S, et al; Crystalline solids; Advanced Drug Delivery Reviews; (48); 2001; pg. 3 – 26; folios 146 - 157.

En consecuencia, la reivindicación 1 no es nueva, según lo divulgado en el documento D1.

La reivindicación dependiente 18 no menciona alguna característica que haga nuevo al compuesto de la reivindicación 1, por lo cual se considera que no es nueva.

Nivel Inventivo (Art18 D486)

La reivindicación 2 consiste en un compuesto cristalino que es tosilato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato.

La reivindicación 8 consiste en un compuesto cristalino que es tosilato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato monohidrato.

La reivindicación 13 consiste en un compuesto cristalino que es tosilato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato dihidrato.

La reivindicación 17 consiste en un compuesto cristalino que es maleato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato.

Comparación de las características técnicas esenciales, con las del Estado de la Técnica:

Características esenciales	D1	D2
Tosilato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato	Ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico (pg. 92, compuesto 539).	Ácido p-toluenosulfónico como ácido para formar sales farmacéuticamente aceptables (pg. 309, fl 143).
Tosilato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato monohidrato		
Tosilato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato dihidrato		
Maleato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato		Ácido maléico para formar sales farmacéuticamente aceptables (pg. 292, fl 143).

El documento D1 se considera el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulga el compuesto ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico.

La diferencia entre la invención, definida en la reivindicación 1 y los compuestos de D1 consiste en divulgan de manera específica sus sales tosilato y maleato y los hidratos de la primera sal. Se aclara, que aunque en las reivindicaciones se encuentran los términos hidrato o de forma cristalina, no están las características técnicas esenciales que peritan afirmar que los compuestos corresponden a formas cristalinas o hidratos del compuesto de la invención.

El efecto que logra el incluir esta característica es que proporciona sales del compuesto ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular como la necesidad de proporcionar sales de el compuesto ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico.

Sin embargo, D2 divulga la utilización de ácido p-toluenosulfónico y ácido maléico para la formación de sales farmacéuticamente aceptables (pgs. 292 y 390; fl 143), correspondientes a la sal tosilato y maleato, respectivamente. Además, D1 enseña que con los compuestos que allí da a conocer, por ejemplo el ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanolco, se pueden formar sales farmacéuticamente aceptables que incluyen sales de adición de ácidos como p-toluenosulfónico y maléico, entre otros (pg. 6, lns 15-31). Además, D2 enseña que el mejoramiento de la velocidad de disolución de fármacos que son ácido o bases débiles poco solubles es uno de los principales motivos para la formación de sales (pg. 19 y 28, fl 137). Además, que la selección y la preparación de una sal adecuada dan la oportunidad de modificar características fisicoquímicas y mecánicas de los fármacos que permiten el desarrollo de formas farmacéuticas con mejor biodisponibilidad, estabilidad, entre otros (pg. 163, fl 142). Asimismo, da a conocer que la elección de una sal en particular está determinada por la acidez o basicidad del grupo ionizable, la seguridad del grupo potencial para la formación de la sal (contra-ión), indicaciones del medicamento, ruta de administración y la dosis, ya que la mayoría de las sales se desarrollan con el fin de aumentar su solubilidad (pg. 167, fl 143).

Por otra parte, D2 también enseña que la formación de hidrato de compuestos es una práctica habitual en el sector farmacéutico, con lo cual también se modifican propiedades fisicoquímicas del compuesto en cuestión como solubilidad, estabilidad, entre otros (pg. 51 – 66, fls 137 -141).

En consecuencia, la persona del oficio normalmente versada en la materia se vería suficientemente motivada para utilizar ácido p-tolueno sulfónico y ácido maléico, de D2, para formar una sal farmacéuticamente aceptable o hidratos de estas con el compuesto ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanolco, divulgado en D1, para llegar así al objeto de las reivindicaciones 2, 8 13 y 17 de la solicitud. Por lo cual se consideran obvias.

Conclusión: las reivindicaciones 2, 8, 13 y 17 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

Puesto que las relvindicaciones dependientes 3, 4, 7, 9, 12, 14, 19 y 20 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

La reivindicación 5 consiste en el compuesto cristalino de la reivindicación 3 que tiene un patrón de difracción de polvo de rayos X que comprende un pico en uno o más de aproximadamente 3.5, 7.0, 8.6, 10.9, 13.5, 14.0, 15.1, 17.3 y/o 20.5 2θ grados.

La reivindicación 10 consiste en el compuesto cristalino de la reivindicación 8 que tiene un patrón de difracción de polvo de rayos X que comprende un pico en uno o más de aproximadamente 3.6, 8.2, 8.7, 13.1, 14.5, 17.5, 18.0, 19.9 y/o 21.4 2θ grados.

La reivindicación 15 consiste en el compuesto cristalino de la reivindicación 13 que tiene un patrón de difracción de polvo de rayos X que comprende un pico en uno o más de aproximadamente 8.6, 9.0, 17.2, 17.8, 18.6, 21.6, 25.2 y/o 26.9 2θ grados.

Comparación de las características técnicas esenciales, con las del Estado de la Técnica:

Características esenciales	D1	D3
Forma cristalina de tosilato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato anhidro	Ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-	Enseña que el fenómeno de polimorfismo (formas cristalinas) es la capacidad

Forma cristalina de tosilato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato monohidrato	1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil) propanoico (pg. 92, compuesto 539).	de cualquier elemento o compuesto para cristalizar en más de un sistema cristalino y es un trabajo habitual en el campo de preformulación farmacéutico.
Forma cristalina de tosilato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato dihidrato		
Patrón de difracción de polvo de rayos X para el anhidrato que comprende un pico en uno o más de 3.5, 7.0, 8.8, 10.9, 13.5, 14.0, 15.1, 17.3 y/o 20.5 2θ grados.	No se menciona	Enseña que la caracterización de formas cristalinas para un mismo compuesto por medio del patrón de difracción de rayos X es un método convencional
Patrón de difracción de polvo de rayos X para el monohidrato que comprende un pico en uno o más de 3.6, 8.2, 8.7, 13.1, 14.5, 17.5, 18.0, 19.9 y/o 21.4 2θ grados.		
Patrón de difracción de polvo de rayos X para el dihidrato que comprende un pico en uno o más de 8.8, 9.0, 17.2, 17.8, 18.6, 21.6, 25.2 y/o 26.9 2θ grados.		

Aproximación método problema-solución para polimorfos (formas cristalinas)

1. Determinación del estado de la técnica más cercano;

El documento D1 se considera el estado de la técnica cercano a la invención definida en las reivindicaciones 5, 10 y 15 porque divulga el compuesto ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico (pg. 92, compuesto 539).

2. Determinación de las diferencias técnicas de la invención con respecto al estado del estado de la técnica más cercano:

La diferencia entre la invención definida en las reivindicaciones 5, 10 y 15 y el compuesto del documento D1, consiste en que revela de manera específica el anhidrato, monohidrato y dihidrato de la sal tosilato del compuesto de D1, caracterizado por picos de difracción de rayos X en polvo.

3. Determinación del efecto técnico obtenido por las diferencias técnicas de la invención con respecto al estado de la técnica más cercano:

Según la descripción de la solicitud efecto técnico que logra la diferencia es proporcionar diferentes formas cristalinas de ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico con mejores propiedades como solubilidad y estabilidad.

4. Establecimiento del problema técnico objetivo a solucionar con base en el estado de la técnica más cercano:

El problema técnico objetivo que pretende resolver esta invención se puede formular como la necesidad de obtener diferentes sales e hidratos del compuesto ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico con mejores propiedades como solubilidad y estabilidad.

5. Comprobación de la solución al problema técnico objetivo de la solicitud:

En ninguna parte de la descripción se encuentra soporte de las propiedades físicas, mecánicas o fisicoquímicas superiores de los hidratos de la sal obtenidos respecto del compuesto del estado del arte. Por lo anterior, no se puede comprobar la solución

al problema técnico objetivo planteado en la solicitud porque no se realiza una comparación técnica de las propiedades del compuesto de la invención y del estado de la técnica más cercano que pueda ser reproducido por la persona del oficio versada en la materia.

Por lo tanto se puede concluir que no existe un efecto técnico probado, porque no se demuestra las ventajas relacionadas con las propiedades físicas, mecánicas y/o fisicoquímicas como solubilidad y estabilidad reclamada en la actual solicitud, con respecto a la forma sólida del compuesto divulgado en D1.

6. Evaluar si habría sido obvio solucionar el problema técnico objetivo por la invención reivindicada:

De tal manera que la solución al problema técnico planteado por la invención reclamada se considera obvia porque la anterioridad D3, ya divulga que las diferentes formas cristalinas, como los hidratos, para un mismo compuesto difieren en sus propiedades físicas como densidad, dureza, índice de refracción, compresibilidad, punto de fusión, entalpía de fusión, presión de vapor, solubilidad velocidad de disolución, y otras propiedades termodinámicas y cinéticas (fl 146, pg. 4), y es además una práctica deseable en la etapa inicial del desarrollo de medicamentos, esto con el fin de hallar la forma más adecuada y estable del activo (abstract).

También es conocido en el campo técnico que los pseudopolimorfos (como los hidratos) son diferentes arreglos estructurales dentro de un cristal de una misma molécula o sustancia en cuya estructura contiene moléculas de solvente (agua), por lo tanto una persona del oficio normalmente versada en la materia, conociendo que en D1 ya se divulga una forma sólida de ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico, y que en D3 se conoce que el fenómeno de la formación de hidratos, esta persona se encontraría motivada a utilizar métodos como los revelados en D3 para identificar y obtener las formas pseudopolimórficas de la molécula en cuestión, que además pueden ser analizadas por métodos de cristalografía conocidos (fl 153, pg. 18). De tal manera, no es posible concluir que la obtención de las formas cristalinas del anhidrato, monohidrato y dihidrato de la sal tosilato reclamadas en la actual solicitud envuelva actividad inventiva, como quiera que la molécula misma ya era conocida con anterioridad; es decir, la formación de hidratos y la identificación de estas formas cristalinas reclamadas mediante difracción de rayos X en polvo, no representa un avance técnico real y tampoco un salto cualitativo a la regla técnica porque el efecto asociado no fue demostrado en la descripción de la solicitud, comoquiera que no se encontró alguna prueba o ensayo que permitiera determinar las ventajas reales de del anhidrato, monohidrato y dihidrato de la sal tosilato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato con respecto a la forma sólida del mismo compuesto conocida con anterioridad, que permita probar, un efecto realmente inesperado asociado a propiedades físicas y/o fisicoquímicas como solubilidad y estabilidad.

En consecuencia, una persona del oficio normalmente versada en la materia, encontraría la sugerencia o motivación suficiente aplicando los métodos y conocimientos que se tiene sobre el pseudopolimorfismo revelados en D3, a la forma sólida de ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico o sus sales divulgada en D1, con el fin de formar el monohidrato o dihidrato de tosilato de (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoato tal como se reivindica. Es más, aún demostrando mejor solubilidad o estabilidad, estos resultados

son esperados, son obvios para la persona versada en la materia, pues la obtención de mejores propiedades con la formación de hidratos es enseñada por D3.

De acuerdo con lo expuesto, las reivindicaciones 5, 10 y 15 no cumplen con el requisito de nivel inventivo (Art. 18).

Puesto que las reivindicaciones dependientes 6, 11 y 16 no mencionan características inventivas adicionales tampoco se puede considerar inventiva.

La reivindicación 21 consiste en un método para preparar una sal cristalina de ácido (S)-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico, que comprende: calentar una solución que comprende ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico y un ácido farmacéuticamente aceptable para proporcionar una sal de ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico; reducir la solubilidad de la sal en la solución bajo condiciones suficientes para proporcionar una sal cristalina de ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico; y aislar la sal cristalina.

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1	D2
Método para preparar una sal cristalina de ácido (S)-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico, que comprende:	Sales de ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico (pg. 92, compuesto 539).	Método para preparar una sal cristalina de ácido maléico (pg. 254-255, fl 145)
calentar una solución que comprende ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico y un ácido farmacéuticamente aceptable para proporcionar una sal de ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico;	No se menciona	Agregar ácido maléico a una solución que contiene el principio activo disuelto en un solvente
reducir la solubilidad de la sal en la solución bajo condiciones suficientes para proporcionar una sal cristalina de ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico; y	No se menciona	Dejar cristalizar el compuesto.
Aislar la sal cristalina.	No se menciona	Aislar la sal cristalina por cristalización.

El documento D1 se considera el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulga el compuesto ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico.

La diferencia entre la invención, definida en la reivindicación 20 y el proceso de D1 consiste en divulgan de manera específica el proceso de obtención de sus sales.

El efecto que logra el incluir esta característica es que proporciona un proceso para obtener sales del compuesto ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanoico.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se

puede formular como la necesidad de proporcionar un proceso para producir sales de el compuesto ácido (S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-metoxibifenil-4-il)etoxi)pirimidin-4-il)fenil)propanolico.

Sin embargo, la formación de sales de principios activos es una práctica común en el sector farmacéutico. Además, D2 enseña que la formación de sales de ácido se realiza por la adición de un ácido al principio activo disuelto en una solución, y que la sal formada se separa por su insolubilidad en el medio en el que se obtiene y que posteriormente se puede aislar de forma cristalina mediante métodos como la cristalización (pg. 250 -257, fls 144 y 145).

En consecuencia, la persona del oficio normalmente versada en la materia se vería suficientemente motivada para emplear los procesos conocidos en el estado del arte para formar sales de ácido, como los que consisten en la adición de un ácido al principio activo disuelto en una solución, posteriormente la separación de la sal formada por su insolubilidad en el medio en el que se obtiene y finalmente, aislarla en forma cristalina mediante métodos como la cristalización, como se enseña en D2, en el compuesto divulgado en D1, para llegar así al objeto de la reivindicación 21 de esta solicitud, por lo cual se considera obvia.

Conclusión: las reivindicación 21 cumple el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

Puesto que las reivindicaciones dependientes 22 – 27 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

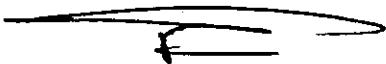
8. Conclusión

Los requisitos de patentabilidad de la presente solicitud están afectados así:

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO2007/089335	1 y 18	Novedad
D1	WO2007/089335	2 – 17 y 19 - 27	Nivel inventivo
D2	Libro	2-4, 7-9, 12-14 y 17, 19-27	Nivel inventivo
D3	Artículo	5, 6, 10, 11, 15 y 16	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

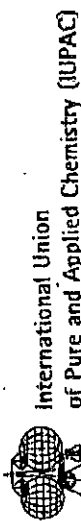
Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha 00 OCT. 2012

Elaborado por: Rubiela Pacanchique Vargas *out*
Revisado por: Jacqueline Alfonso Rezo



International Union
of Pure and Applied Chemistry (IUPAC)

Handbook of

Pharmaceutical Salts

Properties, Selection, and Use

P. Heinrich Stahl, Camille G. Wermuth (Eds.)



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Chapter 2

Solubility and Dissolution of Weak Acids, Bases, and Salts

by Madhu Pudipeddi*, Abu T. M. Serajuddin, David J. W. Grant,
and P. Heinrich Stahl

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1. Introduction

Improvement of dissolution rate of weakly acidic or weakly basic drugs that are poorly soluble is one of the primary reasons for preparation of pharmaceutical salt forms [1-7]. Salt forms have also been used to control drug dissolution [8] [9]. This chapter examines the pH-dependencies of the solubility and dissolution rate of pharmaceutical acids, bases, and salts, and the relevance of these dependencies to the selection of the final form.

nation. Further confirmation of the nature of the excess solid phase may be obtained by powder X-ray diffractometry or elemental analysis. If the excess solid phase is entirely in the free form, the experiment must be repeated with a higher solid to solvent (H_2O) ratio until the excess solid contains the salt. In some cases, complete conversion of the salt to the free form may occur at all solid-to-solution ratios, often leading to formation of a 'gel'.

As a result of the above phenomenon, in some cases, an attempt to make a solution of a 1:1 salt at a concentration well below the solubility of the salt (as estimated from the K_{sp} value) will be unsuccessful due to precipitation of the free unionized form. To further develop this point *Anderson and Flora* [2] defined the term 'stoichiometric solubility'. The stoichiometric solubility of a 1:1 salt is the concentration of salt which can be dissolved in pure H_2O with no formation of a precipitate of the unionized free form. The authors pointed out that the difficulties of low 'apparent solubility' of a salt due to free form precipitation may be encountered in the preclinical stages of development when concentrated solutions in H_2O are required for toxicology testing. However, appropriate pH adjustment should overcome this problem.

3. Dissolution Behavior of Acids, Bases, and Their Salts

The term *dissolution* refers to the overall process by which a solid compound dissolves in a liquid medium, while *dissolution rate* is the kinetic descriptor giving the rate at which the dissolution takes place. The concept of solubility, on the other hand, implies that the process of dissolution has reached equilibrium, and the solution is saturated. Dissolution rate of solids is of paramount importance in the development of pharmaceutical products and quality control. Salt formation is one of the most commonly employed techniques to improve dissolution of weakly acidic or basic drugs.

A number of theories of the dissolution of solids have been proposed [17] [29] [30]. However, the simple diffusion model may be adequate to describe the dissolution behavior of most pharmaceutical solids in aqueous and non-aqueous media. The diffusion layer model (Fig. 7) assumes that a thin film of saturated solution of concentration c_s exists at the interface of the dissolving solid and the dissolution medium. The dissolution rate is controlled by the diffusion rate of solute molecules from this thin saturated film into the bulk solution. The concentration of the solute in the bulk is denoted by c_b . As the distance x increases from $x = 0$ (at the surface of the solid) to $x = h$ (at the beginning of the bulk solution), the concentration decreases from $c = c_s$ to $c = c_b$. Beyond $x = h$, the bulk concentration remains uniform at c_b . In the simplest form of the theory, following *Fick's First Law*, the rate of dissolu-

3.2. Solubility

The process of dissolution involves the breaking of interionic and/or intermolecular interactions in the solid solute, the separation of the solvent molecules from each other to provide space for the solute, and the formation of interactions between the solvent molecules and the solute molecules or ions. The intermolecular bonding forces in solids include *Van der Waals* forces, H-bonds, and ionic interactions. *Van der Waals* forces include dipole-dipole forces, dipole-induced dipole forces, and induced dipole-induced dipole forces (i.e., *London* dispersion forces). H-Bonding is the most directional of the intermolecular interactions. The polarity of a molecule can be expressed in terms of its dipole moment. In general, the solubility of a substance in H_2O increases with increasing dipole moment, other factors, such as melting point, being equal. If the solute in a solution consists of molecules with a small or zero dipole moment, it is said to be nonpolar and the interaction between solute and solvent is dominated by dipole-induced dipole forces and *London* dispersion forces.

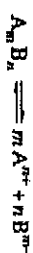
In the case of a salt, the attractive force, F , between oppositely charged particles or ions is expressed by *Coulomb's law*:

$$F = q^+ \cdot q^- / D \cdot d^2 \quad (5)$$

where q^+ and q^- represent the electric charges on the particles or ions, d is the distance between them and D is the dielectric constant of the surrounding medium with respect to vacuum for which $D = 1$.

The ion-dipole interaction is the force that dominates the dissolution of salts in polar solvents. In aqueous solution, the ions are surrounded by (i.e., hydrated by) as many H_2O molecules as possible. The amount of H_2O of crystallization affects the heat of solution. In a crystal hydrate, the ions are already largely hydrated in the crystal lattice and, consequently, the ion-dipole interaction energy liberated on dissolution is considerably less than that of the anhydrous solute during the dissolution process. More considerations of solubility are discussed in *Chapt. 2*.

In the case of a solid salt, for which the stoichiometry is represented by $A_m B_n$, dissolution may be accompanied by dissociation of the ions which causes the ions to separate; thus:



The apparent, concentration-based, solubility product, K_s , is given by:

$$K_s = [A]^{m'} [B]^{n'} \quad (6)$$

In the absence of added ions, the solubility of the solid, defined as the ionic molarity, i.e., the number of moles of ionic charges of each ion dissolved

per unit volume of solution is:

$$C = n[A] = m[B] = (K_s)^{1/(m+n)} \cdot m^{n/(m+n)} \cdot n^{m/(m+n)} \quad (7)$$

If dissolution occurs without dissociation, the solubility, c_s , is directly given by the concentration of the undissociated species, thus,

$$c_s = [A_m B_n] \quad (8)$$

The changes in the energy of the interactions as a solute dissolves is manifested as the enthalpy of solution, ΔH_{sol} , while the standard free energy change, ΔG^θ , is related to the solubility c_s ,

$$\Delta G_{sol}^\theta = -RT \ln c_s \quad (9)$$

where R is the gas constant and T is the absolute temperature. According to the *van't Hoff* equation, the logarithm of the equilibrium constant (e.g., solubility product) is a linear function of the reciprocal of the absolute temperature, as follows,

$$\ln K_s = \ln K_0 - \Delta H_{sol} / RT$$

or

$$\text{or } K_s = K_0 \cdot e^{-\frac{\Delta H_{sol}}{RT}} \quad (10)$$

The solubility of polymorphs is related to their thermodynamic activity, to the escaping tendency of their molecules, and hence to their melting point. For riboflavin [2] a linear relation was found between the log of the solubilities and the melting points of the three polymorphic forms. The thermodynamically stable form at a given temperature and pressure is the form with the lowest free energy and the poorest solubility.

For each polymorph, we may apply the *van't Hoff* Eqn. 10 in the form:

$$\ln c_s = -\Delta H_{sol} / RT + c \quad (11)$$

where c is a constant and the other quantities have been defined above. The solubility curves obtained are shown in Fig. 6. In the case of enantiotropy, there is a transition point at which the solubility of the two polymorphs is identical. In the case of monotropy, the curves do not intersect. However, if a solvent-mediated transition occurs, it would result in spontaneous precipitation of the thermodynamically stable form. Plotting $\ln c_s$ vs. $1/T$ for each modification (polymorph or solvate) allows the determination of the transition point and the calculation of ΔH_{ts} for each modification. The difference of ΔH_{sol} between two forms is the transition enthalpy. The same applies to the transition between a solvate and an unsolvated form. In the case of different hydrates, different transition points can be observed (Fig. 7). This solubility vs. temperature approach is frequently applied for determining the re-

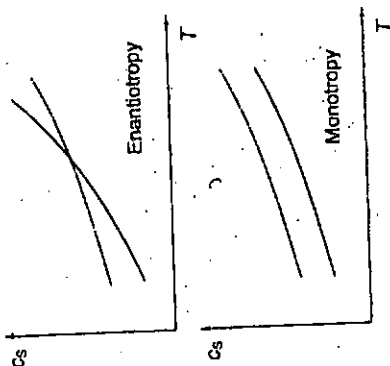


Fig. 6. Plots of solubility c_s vs. temperature T (solubility curves) for enantiotropic and monotropic systems

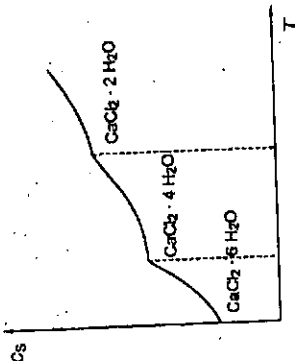


Fig. 7. Solubility curves of solvates, exemplified by the dihydrate, tetrahydrate and hexahydrate of calcium chloride

lationship between the polymorphs of anhydrous forms and even for solvates or hydrates [33].

When comparing the solubilities of salts, the impact of polymorphism has to be taken into account as for nonelectrolytes. Dissolved concentrations should be measured as a function of time when determining the solubility of the different forms because transformation during the measurement may occur. Furthermore, for proper interpretation of the results of solubility measurements, the temperature has to be maintained constant and accurately defined.

Solubility can be measured by phase solubility analysis, where an excess of the solid remains in equilibrium with the saturated solution [34–37]. The solution is analyzed by gravimetry, high performance liquid chromatography (HPLC), or spectroscopy. High throughput methods of solubility determination have been developed based on the measurement of the appearance of the solid phase by nephelometry or by ultraviolet spectrophotometry [38] [39]. In all solubility determinations, soluble impurities can significantly influence the measured solubility. For example, in the case of a single-point measurement on a supersaturated solution, if the relative amount of a drug substance of 98% purity is 20 mg/ml in the suspension, 0.4 mg of the impurity may be dissolved. If the solubility of the drug substance is 1.0 mg/ml, an apparent solubility equal to $1.0 + 0.4 = 1.4$ mg/ml will be found, whereas, if the solubility of the drug substance is 0.5 mg/ml, the apparent solubility will be $0.5 + 0.4 = 0.9$ mg/ml. This discussion highlights the relevance of the conditions used by comparing solubilities. Figs. 8 and 9 emphasize the influence of both impurities and polymorphism [35].

In the case of salts, the relationships are more complex because a salt may be partially dissociated. By measuring the solubility in buffers, the possibility of the salting-out effect (and less frequently, the salting-in effect) must also be considered. For polybases or polyacids, there are pH domains of stability for each species, and the pH-solubility profiles should be measured. The pH profile of a base or an acid may be calculated on the basis of the

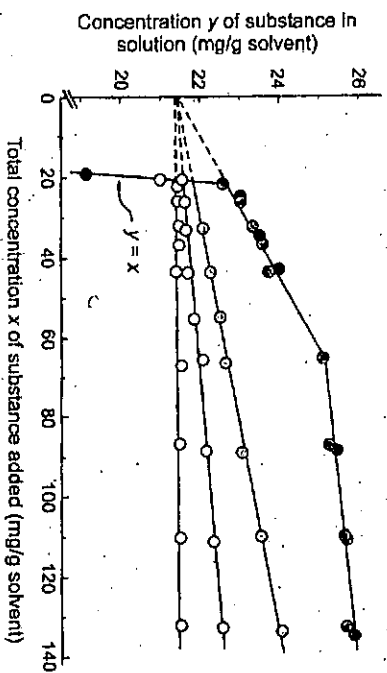


Fig. 8. Phase solubility analysis of 7-(2-hydroxypropyl)theophylline in AcOEt, showing the influence of impurities in increasing the solid solubility. Increasing impurity content of the five samples used in the study is indicated by the increasing gray intensity of the data points. Below saturation, the data points lie on line $y = x$ indicating complete dissolution of the sample added to the solvent. (redrawn from [35]).

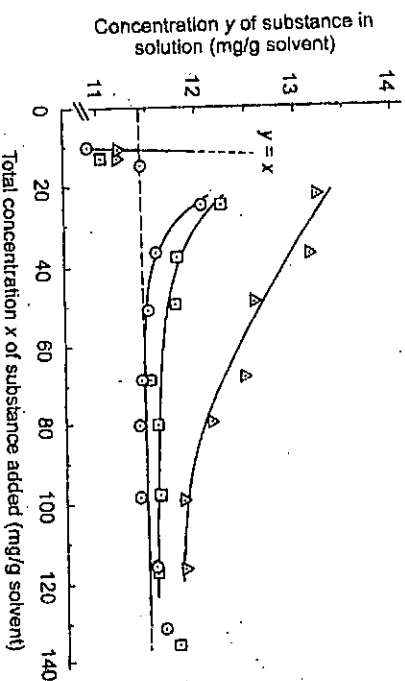


Fig. 9. Phase solubility analysis—transformation of a higher soluble into a less soluble form depending on the time allowed for equilibration. Δ : 20 h; $\square$: 63 h; $\circ$: 115 h (redrawn from [35]).

known intrinsic solubility (the solubility of the undissociated species) and the pK_a (see Chapt. 1 and 2) [38] [39]. By means of recently introduced automated titration methods, the pH profile of the solubility can be obtained quite fast (see Chapt. 2). The pH domains of stability, mentioned above, are different in H_2O or in water + liquid media used as pharmaceutical formulations, as well as in the solvent(s) of crystallization as defined by the phase diagrams of each species with the solvent. Here again, polymorphism and pseudopolymorphism must also be taken into account. Generally, the solubility in H_2O increases in the rank order: hydrate < anhydrate < solvated form, but exceptions exist, especially when the transition temperature lies below the ambient temperature. In organic media, e.g., in alcohols, a hydrated form is usually more soluble than the anhydrous form. For nedocromil magnesium, the rank order of the pentahydrate, the heptahydrate, and the decahydrate was not exactly the same for the solubility and intrinsic dissolution rate (IDR) [40], because the metastable higher hydrates transform to the stable pentahydrate during contact with H_2O in the solubility measurements.

3.3. Dissolution Rate

The dissolution rate of powders is often measured by the flow-through cell method as described in Chapt. 2. When comparing different salts, this

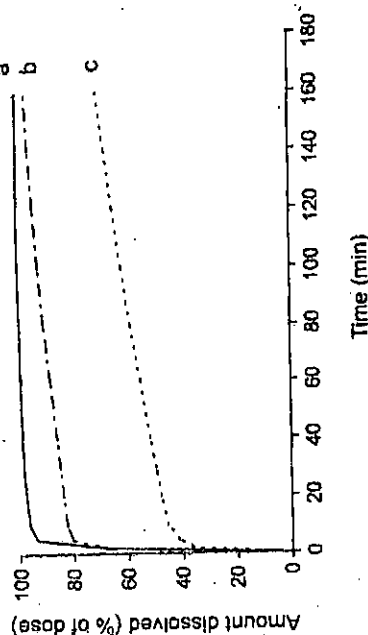


Fig. 10. Influence of particle size on the dissolution process of reserpine hydrochloride in 0.1M HCL. a) Particle size: 99% <49 μm, 90% <67 μm; b) particle size: 99% <67 μm, 90% <120 μm; c) particle size: 99% <120 μm, 90% <120 μm.

method has the disadvantage that the dissolution rate depends on the particle size. On the other hand, this dependency is utilized for a given form to evaluate the effect of particle size, as demonstrated in Fig. 10 [41]. As for solubility measurements, the pH of measurement and the salt effect of the buffer have to be taken into consideration.

The transformation of an anhydrous form into a hydrated form and the transformation of a salt into the free base or acid is often observed. Fig. 11 is an example of the observed dissolution rates in the case of transitions during the course of the experiment:

- I: The anhydrous form dissolves without change;
- II: the anhydrous form transforms into a hydrate;
- III: instantaneous change of the anhydrous form into the hydrate and dissolution of the less soluble hydrate.

The difference, *b*, between the curves II and III results from the concurrence of the dissolution process and the transformation process.

Fig. 12 deals with a drug substance with polymorphic behavior [41]. The dissolution rate of two samples of the drug substance, representing two polymorphs of almost the same particle size, is reflected in the dissolution rate of the drug product as capsules.

The dissolution rate per unit surface area, termed the intrinsic dissolution rate (IDR; see Chapt. 2) is independent of the particle size. In the 'disc' method,

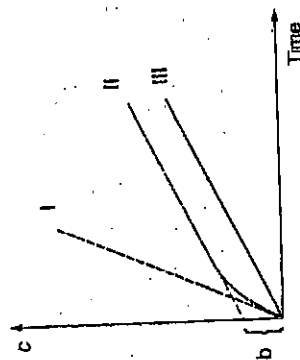


Fig. 11. Plots of the dissolution of a compound that forms an anhydrous and a hydrate. I) Anhydrous dissolves without phase change; II) anhydrous dissolves followed by phase change into the hydrate; the hydrate, being less soluble, dissolves more slowly; III) instantaneous phase change into the (less soluble) hydrate occurs, followed by the dissolution of the hydrate.

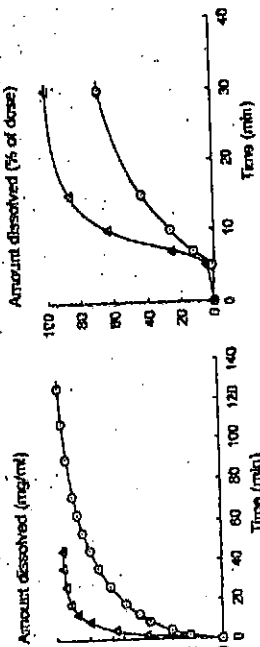


Fig. 12. Dissolution curves (left) of two erythrosine modifications (A and B) of a drug substance and the corresponding curves (right) the drug products (capsules). Δ : polymorph A; $\circ$: polymorph B.

ed, the powder is compressed by a punch in a die to produce a compact disc or tablet. Instruments are now commercially available for this purpose [42]. Only one face of the disc is exposed to the dissolution medium, and the cumulative amount dissolved per unit surface area is determined by ultraviolet spectrophotometry until 10% of the solid is dissolved. The slope of the plot of mass dissolved per unit surface area vs. time gives the IDR in appropriate units, e.g., mg/min/cm². If a change in the slope is observed during the course of the experiment, then a change in the solid phase exposed to the solvent occurs during the experiment. A solid phase with different state of ionization (e.g., the unionized form) as well as a polymorphic change can occur. For example, the initial dissolution rate for a drug substance was 0.05 mg/min/cm².

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After several minutes, the dissolution slowed down in order to continue with the lower rate of 0.01 mg/min/cm², resulting from the formation of a hydrate. A separate measurement of the IDR of the hydrate indeed reproduced the same rate of 0.01 mg/min/cm².

3.4. Heat of Solution

The enthalpy of the solution process, i.e., the heat of solution, ΔH_{sol} , can be calculated from the temperature dependence of the solubility as discussed in Sect. 3.2. This method has been widely used, for example, for cloxacetyl base and its hydrochloride [43]. However, *Hollenbeck* [44] and *Burger* [23] consider that the slope of the linear relation predicted by *Eyring* [10] is too variable, and is subject to systematic errors of several percent due to nonideality of the saturated solution, resulting in inaccurate determination of both the heat of solution and the transition point of two forms. The heat of solution of substances can be measured directly by solution calorimetry, which clearly provides more significant values [18] [45]. The difference between the heats of solution of the two polymorphs is equal to the transition enthalpy of the polymorphs at temperatures close to ambient. The results provide an alternative to DSC for the discrimination between enantiotropy and monotropy, when the substance decomposes upon melting. Quantitative analysis of polymorphs, solvates, and amorphous forms in mixtures has been performed using solution calorimetry. Studies of polymorphism by solution calorimetry have been reviewed [18].

3.5. Interaction with Water Vapor

Water vapor is an omnipresent component of the atmosphere. The vapor pressure of H₂O at different temperatures has been tabulated. At a given temperature, the ratio, actual water vapor pressure/saturated water vapor pressure at that temperature is termed the *relative humidity* (r.h.), given as percentage of saturation. The environmental humidity depends on the weather and climatic zone and may vary from 10 to more than 75%. This variability has to be taken into account when determining the storage conditions of a salt candidate.

When a solid is exposed to a gas, some of the gas molecules, termed the *adsorbate*, become attached to the surface of the solid, termed the *adsorbent*. This process, which is divariant, is called *adsorption*. When water vapor is adsorbed, the H₂O molecules may undergo any type of interaction (*Van der Waals*, ion-dipole, and specific H-bonding interactions) with the functional groups on the surface of the adsorbent; they may cause swelling of hygro-

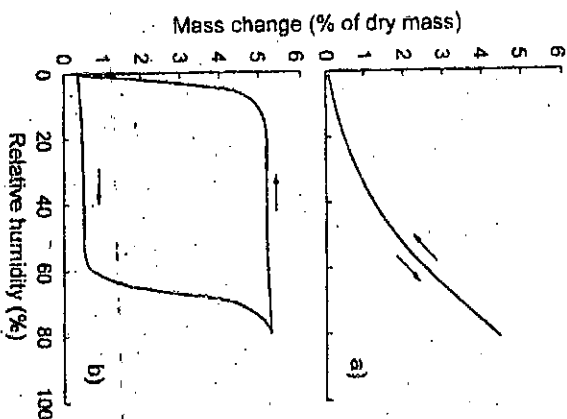


Fig. 13. Examples of water sorption/desorption isotherms. a) Sorption/desorption is reversible; b) Desorption exhibits hysteresis.

philic polymers, may induce a phase transformation of the adsorbent to form a solid hydrate, or may cause a hygroscopic adsorbent to form a pool of saturated solution by the process known as *deliquescence*. Water sorption and desorption isotherms of drug substances should be determined, especially if the solid undergoes a phase change. Hygroscopicity and sorption kinetics have been reviewed [46] [47], and the use of water sorption isotherms for comparison of salt candidates has been discussed [48].

In the case of certain drugs and excipients as adsorbents for water vapor, the sorption and desorption isotherms may not coincide, giving rise to hysteresis as illustrated in Fig. 13. Limiting the sorption of water vapor from ambient air is crucial for maintaining the quality of some pharmaceuticals during their manufacture. The behavior of drug substances at different temperatures and humidities for different climates is generally studied by gravimetry. The humidity for gravimetric studies of water sorption and desorption can be controlled by saturated salt solutions [49] or by humidification of a stream of air or N₂ to which the solid sample is exposed. In the so-called "dy-

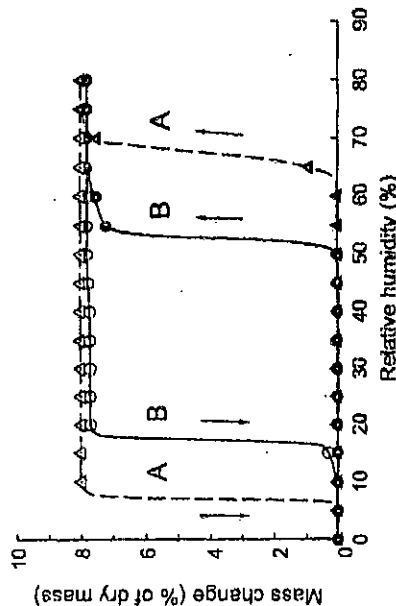


Fig. 14. Two polymorphs (A and B) transforming into the same hydrated form. The metastable form B takes up H₂O at lower relative humidity than the stable form A. The hydrate form loses H₂O at r.h. values below 20%.

namic water sorption' system [50], the sample is placed on a microbalance and exposed to a continuous flow of air or N₂ with constant, predetermined relative humidity. By these methods, samples as small as 1 mg can be accurately studied.

Solid-state hydration may occur leading to the formation of hydrates. X-ray diffractometers may be equipped with special sample cells for exposing the sample to controlled temperature and humidity. The structural changes in the solid can be monitored, and the extent of their reversibility can be studied. The powder X-ray diffraction patterns collected during heating allow dehydration steps, or other desolvation steps, to be followed efficiently. Because sorption and desorption phenomena are accompanied by heat exchange, microcalorimetric techniques are valuable for monitoring purposes [51] [52].

Fig. 14 shows the sorption isotherms of two crystalline modifications of a drug substance both of which transform into a hydrated form at 25°C [53]. Under ambient conditions, the thermodynamically stable form is less hygroscopic. The critical humidity at which the mass changes abruptly, corresponding to the humidity at which the formation of hydrates begins, depends upon the temperature. The higher the temperature the lower is the critical humidity. The formation of theophylline monohydrate at the surface of tablets containing anhydrous theophylline is an example that has been thoroughly studied [54-56].

Different polymorphs usually behave differently [18]. A metastable polymorph may transform into a crystalline hydrate, whereas the stable polymorph may not (e.g., see Table 3). Solvates may transform into hydrates by direct displacement of a solvent guest by H₂O. This process suggests a method for obtaining hydrates that cannot otherwise be manufactured [57]. When several hydrates are formed, the kinetics of the dehydration processes may be different from the kinetics of the hydration step, as in the case of nafazagel hydrochloride [58] for which the sorption of H₂O occurs in two steps via the hemihydrate. The resulting monohydrate loses H₂O in one step, yielding the anhydrate. Due to the different kinetics of their transformations, the anhydrate, hemihydrate, and monohydrate can co-exist for an appreciable period of time. Hydrates have been the subject of several reviews (e.g., [59]).

Fig. 15 illustrates the complexity of hydration and dehydration processes in the water-sorption isotherms of three salts of a basic drug [41]. The hydrogen malonate salt takes up moisture reversibly corresponding to a weight change that exceeds 22% at saturation. The hydrogen tartrate salt is also hygroscopic exhibiting a slight hysteresis. For the hydrochloride salt, a strong hysteresis is observed due to the formation of a stable hydrate. The base and the hydrogen maleate salt were not at all hygroscopic. This example illustrates the importance of the study of the hygroscopicity of salt candidates.

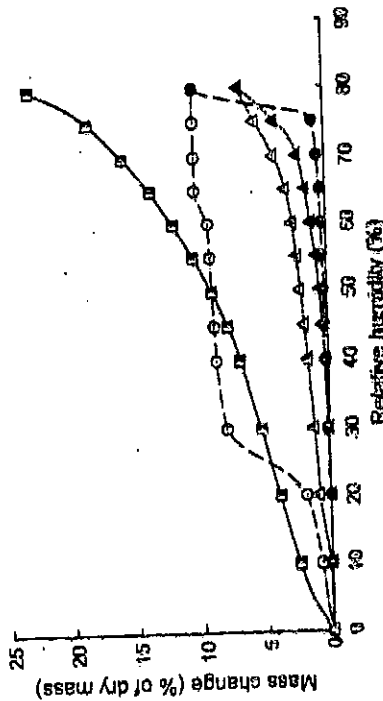


Fig. 15. Water vapor sorption isotherms of three salts of a basic investigational compound. O: Hydrochloride, Δ: hydrogen tartrate, □: hydrogen malonate (sorption and desorption without hysteresis); black symbols: absorption; empty symbols: desorption.

3.6. Density

For solids, different expressions of density have been defined and are considered to be of practical importance for powdered solids. The *tapped density* and the *bulk density* (also called *poured density*) describe the *tapped* properties of a powder and are an indirect measure of the flow properties of the powder resulting from the distributions of particle size, shape, and surface area. On the other hand, the *true density* as a theoretically derived parameter depends on the packing of the molecules in the crystal structure. It is determined by the volume of the unit cell, the number of molecules contained therein, and the molecular weight. Thus, the true density can be calculated if the crystal structure has been determined by X-ray analysis. Experimentally, the true density is measured in a gas pycnometer with He as displacement gas, as described in USP XXIV. According to one of the four conditions of measurement the more densely packed form is the more stable form at 0 K. For example, the densities of three crystalline modifications of the purine derivative, MKS 492, are 1.422, 1.411, and 1.400 g/cm³ [60]. A complete study of the polymorphic behavior of MKS 492 demonstrated that two less dense forms are monotropic in relation to the crystalline modification with the highest density. However, while the density rule is obeyed by MKS 492, it fails for some polymorphic systems. Of the four thermodynamic rules, the heat of fusion rule and the heat of transition rule are found to be more reliable.

3.7. Morphology

Crystal morphology is an important consideration when selecting a salt form. Generally, needle-shape crystals are not desirable because of their poor flow properties [61]. Therefore, it is usual to examine and to compare the crystals under a magnifying glass, light microscope, or scanning electron microscope (SEM). The microscopic techniques have been augmented by image analysis for comparing the morphology of different salts [62] [63]. Morphology of anisotropic crystals may be modified by the conditions of crystallization (crystal engineering) [65].

4. Kinetic Aspects

If phase transformations were based solely on thermodynamic rules, stable crystal forms should be obtained quite easily. However, kinetic factors

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cause metastable states to be encountered. A considerable activation energy barrier may have to be overcome in moving from a metastable state to a stable state. The activation energy, E , is related to the rate constant, k , of the transformation by the well-known Arrhenius equation:

$$k = A e^{-E/RT}$$

(12)

where A is the frequency factor, which is related to the entropy of activation according to Eyring and Polanyi, and R and T have been defined previously (Eqn. 9). The activation-energy barrier may be reduced by catalysts, impurities, and/or crystal defects. Most transformation processes in the solid state involve nucleation, progression of a growth zone, and regression of an unreacted core. When comparing the stability of solids during storage, many factors play a role, such as temperature, particle size, and regression of an unreacted core. The product, activation energy for the change, and diffusion of the molecules.

The influence of nuclei (seed crystals) of the stable form in a sample of a metastable form is demonstrated in the following example. For the substance exemplified in Fig. 12, two batches of the metastable form were stored under different conditions for stability testing. The DSC scans showed that the second batch contained traces of the stable form, whereas no stable form could be detected in the first batch. After 5 years, the second batch had transformed to the stable form at a rate that depended on the temperature, whereas no transformation of the metastable form to the stable form was observed in the first batch at all storage temperatures. After storage of the second batch for 3 years at 30 °C, 50% of the stable form was found by X-ray diffractionometry and by IR spectroscopy [41] [64]. However, no trace of the stable form was found by X-ray diffractionometry in all samples of the first batch. Samples of the second batch, when stored in a deep-freezer for 5 years, contained ca. 5% of the stable form (estimated by X-ray diffractionometry). The different behavior of the batches is explained by the presence of the small amount of seed nuclei of the stable form in the second batch. This example emphasizes the need for sensitive methods to differentiate and to detect polymorphs in mixtures.

Transformations may be accelerated by the presence of a solvent, such as H₂O. Solvent-mediated transformations occur by a continuous dissolution-crystallization or granulation. Hydrates may be formed by this process in mixtures involving moisture. Solvent-mediated transformations may occur when measuring solubilities, such that, after some time, recrystallization of a more stable state may be complete (Fig. 16).

Table 2 summarizes solubility measurements on four forms, A, B, C, and D, of MKS 492 as functions of time and at different temperatures [60]. After

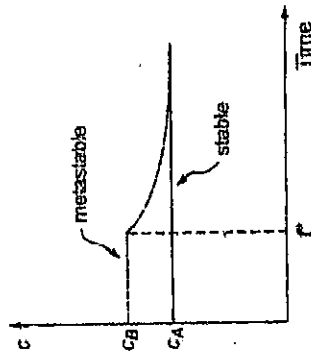


Fig. 16. Concentration vs. time plots showing the possible solubility behavior of stable (A) and metastable (B) polymorphs or pseudopolymorphs, respectively. At the time indicated t^* , the saturated solution of the metastable form, which is supersaturated with reference to the stable form, starts to crystallize, thereby approaching the concentration of the lower solubility of the stable form. Crystallization may occur by spontaneous nucleation or can be induced by seeding with the stable form.

Table 2. Solubility Measurements of MCS 497 Polymorphs, and of Mixtures of Each Polymorph with the Stable Form B, in H_2O [50]

Temperature [°C]	Time [min]	Polymorphs				Mixtures			
		A	B	C	D	A+B	C+B	D+B	
20	10	2.71	1.71	2.16	1.77	1.57	1.67	2.15	
	20	2.76	1.62	2.02	1.76	1.55	1.60	2.00	
	40	2.77	1.63	2.07	1.87	1.60	1.67	1.65	
30	10	2.96	1.76	3.03	2.21	1.86	1.77	2.22	
	20	2.98	1.58	2.76	2.34	1.70	1.59	2.04	
	40	3.00	1.59	2.82	2.26	1.68	1.66	1.96	
40	10	3.56	1.61	4.51	2.36	1.69	1.58	1.60	
	20	3.61	-	4.31	-	-	-	-	
	40	3.58	-	4.63	-	-	-	-	

equilibration, the aqueous phase was analyzed. Form B is the least soluble form and is, therefore, the most stable form. After 10 min or 40 min, no obvious differences were observed among the measurements. The same experiment was repeated with forms A, C, and D, each containing some of the less soluble form B. The decreases in the solubilities demonstrate the influence

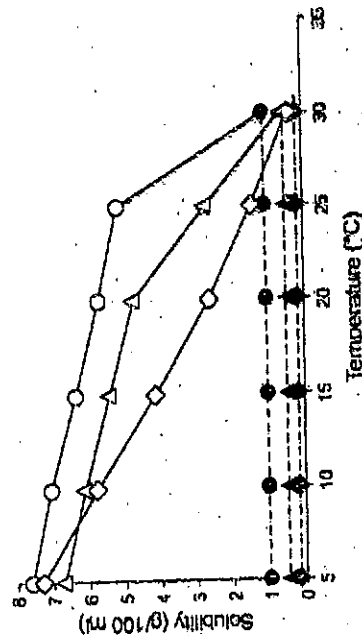


Fig. 17. Influence of temperature on the kinetics of a solvent-mediated transformation in formulations of an investigational drug substance in two crystalline modifications, monitored by the concentration of dissolved substance after one day of storage. Full symbols and broken lines: stable form; hollow symbols and full lines: metastable form; soybean oil: Mglyd 812 and soybean oil.

of seeds of the stable form, which accelerate the solvent-mediated transformation [60].

The influence of temperature on the phase transformation is demonstrated by the example shown in Fig. 17 for an investigational drug of which the stable crystalline form was obtained for the first time only after storage of liquid formulations [41]. A great difference in solubility between the stable and the metastable forms has been observed in this case. In suspensions prepared with the three solvents, it was found that the higher the storage temperature is, the faster the solubility decreases, departing from the initially high value of the metastable form. The rate of transformation is accelerated by increasing the temperature. The transformation is almost complete after one day in suspensions held at 30 °C, whereas the concentrations of substance in solution remain unchanged after a further week of equilibration. This example illustrates that the identification of the stable form of a drug substance and the knowledge of its solubility facilitate the prediction of the physical stability of a pharmaceutical formulation.

There is a metastable zone of supersaturation with respect to the stable polymorphic form, which is important in crystallization processes driven by cooling (Fig. 18). If the solubility curve of a metastable form is in the metastable zone, the first crystal obtained will be the metastable form. Thus, this metastable form will crystallize, and the stable form will not appear, if the

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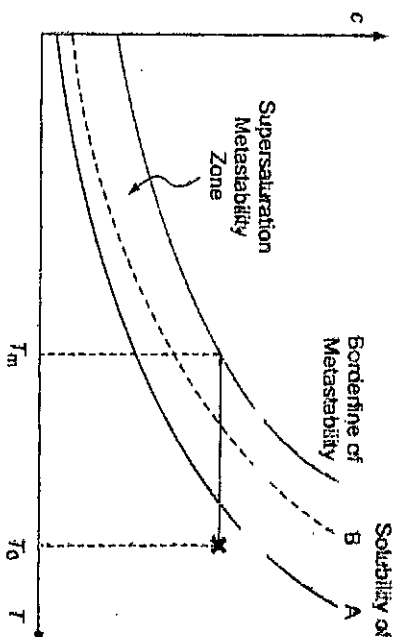


Fig. 18. Crystallization from supersaturated solution by cooling. Plot of solubility c , wt. percentage of two polymorphs, stable A and metastable B with the solubility curve of form B located within the crystallization metastability zone. Cooling starts at *.

transformation into the stable form is limited by the kinetics of dissolution and growth [65] [66]. Difficulties in obtaining crystals of a particular form, which were obtained at an earlier time are discussed by *Dunitz and Bernstein* [67].

Amorphous or partially amorphous solids may result from very rapid precipitation, drying, milling, freeze drying, or shock cooling from the melt. Often, the presence of seeds of a crystalline form in the amorphous bulk represent a 'memory' of the crystalline state especially after drying solvated forms. Amorphous substances are generally hygroscopic; they have high solubility and bioavailability, and good tableting properties, but are difficult to mill. Amorphous forms are usually less stable chemically and physically due to their molecular mobility; they tend to transform into crystalline forms upon storage, humidification, and heating.

Crystallization of an amorphous solid proceeds generally fast at temperatures above the glass transition temperature, which is depressed by the presence of H_2O . The amorphous form may crystallize into one or another of different polymorphs depending on the temperature and humidity, as was observed in the case of indomethacin [7] [68]. The amorphous content of micronized solids is important, because recrystallization produces agglomerates and consequently increases the particle size, as demonstrated by *Ozols* [69]. The hygroscopicity of buprenorphine sodium is related to the amorphous content [70].

4.3.5. Basic Physicochemical Properties of the Hydrobromide RPR200763H
4.3.6. Discussion of Results

5. Optimization - Second Phase: Drug-Substance Form for Preclinical Development
6. Optimization - Third Phase: Drug-Substance Form for Clinical Development
7. Optimization - Fourth Phase: Drug-Substance Form for Marketing

REFERENCES

1. Introduction

Until ca. 15 years ago the drug discovery processes adopted by pharmaceutical companies involved the screening of large numbers of compounds that had been synthesized by the company's research laboratories over many years. Alternative sources of compounds were also available to some companies from university laboratories, or as extracts from a multitude of natural sources. In the last 15 years, there has been a rapid change towards the use of combinatorial-chemistry techniques which are now being used routinely to prepare many thousands of compounds per year. As described in *Chapter 6*, these compounds are generally dissolved in dimethyl sulfoxide (DMSO) to enable screening for activity in an enzyme- or receptor-based assay system. Initial screening usually involves a highly automated 'high throughput screen' (HTS). If the number of 'hits' found is large, the numbers are usually refined by a more involved and specific 'low throughput screen' (LTS) and other selection processes, until a manageable number of 'leads' is available. Often, several of these 'leads' show only moderate activity, and further structural refinement and optimization is invariably necessary, using a mixture of computational techniques and intuition by the chemistry team, until a smaller number (usually 1-5) of highly active potential 'candidates' remain.

These 'candidates' are usually relatively impure free bases, free acids, or neutral molecules, and are often amorphous. Because they are dissolved and stored in DMSO prior to being used in screening procedures, there is no need to ensure that they are crystalline. Also, the preparation of any salts of these compounds is rarely necessary as the neutral/covalent molecule usually exhibits better solubility in both DMSO and the assay system to be used by the pharmacology group. As can be seen readily, these new procedures favor lipophilic molecules, whereas the majority of marketed drug substances are hydrophilic. The need for water-soluble candidates for use in drug development and the vast majority of dosage forms has been recognized [1-4] for many years before the advent of 'combinatorial chemistry'.

Because drug substances are designed to bind with a receptor, enzyme, protein *etc.*, they normally possess several functional groups capable of hydrogen bonding. Some of these functional groups may give potential for self

within an acceptable range of 3–10 for intravenous solutions) should be monitored to help ensure that the formulation will be well tolerated.

Manipulation of drug substance solubility by selection of salts may also be employed to modify the pharmacokinetic profile of the drug (e.g., benzathine penicillin and insulin zinc complexes used in parenteral formulations). Salt formation may be also advantageous where the melting point of the active moiety is low, and it is necessary to mill or micronize the active ingredient to achieve adequate homogeneity. A suitably stable salt may have a melting point that is 50–100 °C higher than that of the free acid or free base. Also, being more ionic, the crystals are likely to be less plastic and more easily deformed by brittle fracture.

Chapter 6 describes some of the theoretical aspects behind salt formation. This chapter describes some of the procedures adopted by the *Preformulation* team at the *Aventis Pharma* (previously *Rhône-Poulenc Rorer*) *Dagham Research Centre* (DRC). These procedures are similar to a systematic approach proposed by Morris *et al.* [3] but were devised empirically with one aim of how to obtain the maximum amount of information from the minimum amount of precious candidate batch. The outline of the process is shown in the Figure.

Where possible, we prepare a range of salts for each new candidate and compare their properties in our preformulation program, which is designed to give us the relative suitabilities of each one. Only one salt form is developed, and its properties should be appropriate to the primary route of administration and the intended dosage form proposed for initial marketing. The properties of the drug substance required for one dosage form may be quite different from those required for another. An understanding of the influence of drug substance, or its salt, properties on the finished product is essential to ensure selection of the optimum salt form. Some of these aspects are dealt with in more detail in Chapter 4. The procedures demonstrated here are also described in outline form in [5].

2. Salt Formation – Is It Necessary, Is It Feasible?

Each new molecule synthesized within the *Discovery Chemistry* group has an entry prepared in the compound database. Among the first information added as an entry into this database for each compound is the calculated pK_a value of each ionizable group in the molecule [6–9]. Another early entry is the calculated $\log P$ value. Furthermore, a small amount of material is made available, the values are determined experimentally on 1–2 mg of sample by potentiometric titration (e.g., *Sigma Model Glipka apparatus*, *Sigma Analytical Instruments Ltd.*), and the values determined experimentally

formation and thus increased hydrophilicity. This salt formation also gives the pharmaceutical chemist and formulation scientist the opportunity to modify the physicochemical (e.g., melting point, hygroscopicity, chemical stability, dissolution rate, solution pH, crystal form etc.) and mechanical (e.g., hardness, elasticity etc.) characteristics of the potential drug substance and to develop dosage forms with good bioavailability, stability, manufacturability, and patient compliance.

For weakly basic drug substances, salts of an inorganic acid (e.g., hydrochloride, sulfate or phosphate), a sulfuric acid (mesylate, esylate, or isethionate), a carboxylic acid (acetate, maleate, or fumarate), a hydroxy acid (citrate or tartrate), or possibly an amino acid (arginine or lysine) could be considered. In the past, hydrochloride salts have often been the first choice for weakly basic drugs, since, as a consequence of the low counter-ion pK_a , salts can nearly always be formed, and recrystallization from organic solvents is normally straightforward. However, the potential disadvantages of hydrochloride salts include unacceptably high acidity in formulations (e.g., parenteral products), the risk of corrosion of manufacturing plant and equipment, less than optimal solubility due to the risk of salting out, and the potential for poor stability, if the drug is acid labile and hygroscopic [2].

Historically, prior to the severe regulatory constraints that abound today, it had been possible to develop the neutral species (free acid or free base) together with one or more different salts. These different chemical forms were often used for specific purposes. An excellent example of this concerns the phenothiazine drug substance, prochlorperazine. The free base was used in suppositories, as it was the most lipophilic form, the mesylate salt was used in injections and in sachets for the preparation of an oral solution, whilst the less soluble maleate was used for the manufacture of tablets.

Occasionally, salts were also prepared in former times to decrease drug substance solubility for use in suspension formulations where very low solubility is necessary to prevent 'Ostwald ripening', for taste masking (e.g., dextropropoxyphene napsylate suspension), or to prepare an extended release product. Emmonate salts have been used in suspension formulations to increase the duration of action (e.g., chlorpromazine emmonate). On some occasions, the selection of a salt with only modest aqueous solubility may be more suitable for use in tablet products prepared by wet granulation, since the use of highly soluble salts can be detrimental to the granulation process. Depending on the dose required, aqueous solubilities in the range of 0.1–1.0 mg/ml will normally be sufficient to satisfy the dissolution requirements for standard, solid, oral dosage forms of drugs with good-to-moderate potency. However, for parenteral solution products, higher solubilities, perhaps 10 mg/ml or greater, depending on the required dose and dose volume, may be required. For parenteral formulations, the pH of solution (normally

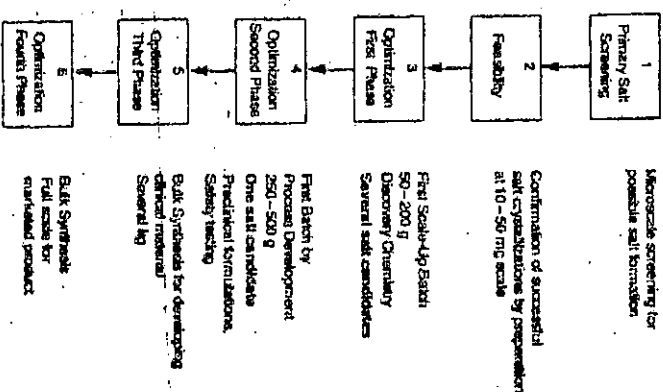


Figure. Process of salt selection and optimization

ly are again entered into the database. The log P value is usually determined concurrently by chromatography using an octanol-loaded HPLC column [10].

Synthesis of a working quantity of each new candidate is rarely undertaken at a scale that exceeds 5 g; the primary synthetic aim is to provide sufficient compound for activity screening in both cell and animal models. Once activity is confirmed in these screens, the material remaining from this first batch is usually further sub-divided across a small number of Departments. The amount of drug substance made available to Physical Chemistry and the Formulation teams together rarely exceeds 1 g and is often less than 0.5 g.

With the pK_a value of each ionizable group in the molecule known, either by experimental determination or by calculation, a list of potential salt forming agents (counter-ions) can be selected, for each candidate, based on

lists that are available in the literature [2][11-13] (see also the tables in the Appendix). Based upon studies on numerous candidates over more than 10 years, it is our considered opinion that, for the formation of a stable salt, there should be a minimum difference of ca. three units between the pK_a value of the ionizable group and a possible counter-ion. This is especially so when the drug substance is a particularly weak acid or base where disproportionation back to the component parts often occurs in solution. This then results in precipitation of the free acid or free base. Occasionally, an exception may be found where a salt has an acceptable stability, despite there being a smaller difference in the pK_a values.

To maximize the amount of data obtained from small quantities of candidate drug substances, micro and semi-micro techniques have been developed and are used regularly within our groups. A microplate technique has been devised by the *Averis Physical Chemistry* group for rapidly and qualitatively screening whether solid salts can be prepared. This involves dissolving ca. 50 mg of sample in a suitable, volatile solvent and adding a fixed volume of this solution, containing ca. 0.5 mg of sample, into each glass microplate well. Concentrated solutions of each potential counter-ion in equimolar proportion, or (if the molecule could form double or triple salts) other appropriate stoichiometric ratios, are prepared, and a few microlitres of each is added sequentially to each well. Thus, all the wells in line 1 (x -direction) will contain the same combination of sample and counter-ion 1; all the wells in line 2 contain the same combination of sample and counter-ion 2, etc. Different, potential crystallizing solvents can be investigated systematically in the y -direction. The wells are inspected using an inverted microscope (Leica, model DM/RB) at regular intervals for the appearance of crystals or solid. Occasionally, crystallization can be promoted by evaporation of any excess solvent in some wells using a slow stream of dry N_2 gas.

Once the possible combinations of counter-ion and solvent(s) are identified, studies at a slightly larger scale (usually 10-50 mg) can be initiated to confirm the formation of a crystalline salt or an amorphous solid and initiate a study of any polymorphism. These studies give a lead to the chemist and can help in the identification of future problems. Melting points are determined by hot-stage microscopy, and samples showing evidence of hygroscopicity can be evaluated using a dynamic vapor sorption (DVS) analyzer (Model DVS-1, Surface Measurement Systems Ltd). There is usually sufficient material for a preliminary powder X-ray study using an environmental chamber (model DB Advance, Bruker Instruments) to qualitatively determine the presence of crystalline or amorphous material. Frequently, these studies can also give information on the existence of solvates and hydrates, especially if differential scanning calorimetry (DSC, Mettler Toledo DSC, model 820), thermal gravimetric analysis (TGA, Mettler Toledo TGA, model 820),

Table 1. Classification of Common Pharmaceutical Salt Formers

Salt Formers	Examples
<i>Anions</i>	
Inorganic acids	Hydrochloride, Hydrobromide, Sulfate, Nitrate, Phosphate
Sulfonic acids	Mesylate, Etylate, Isobutylate, Tosylate, Napsylate, Stearyl
Carboxylic acids	Acetate, Propionate, Malate, Benzoate, Salicylate, Fumarate
Amino acids	Glutamate, Aspartate
Hydroxy acids	Citrate, Lactate, Succinate, Tartrate, Glycolate
Fatty acids	Hexanoate, Octanoate, Decanoate, Oleate, Stearate
Acids for insoluble salts	Panmoate (= submoate), Bitartrate (e.g., Polystyrene sulfonate)
<i>Cations</i>	
Metallic	Sodium, Potassium, Calcium, Magnesium, Zinc
Organic Ammonium	Triethylamine, Ethanolamine, Triethanolamine, Methylamine, Ethylene diamine, Oxidine
Cationic amino acids	Arginine, Lysine, Histidine
Bases for insoluble salts	Proxaline, Benzoamine

and hot-stage microscopy are also used in the evaluation process. Most of these studies can be accommodated, if care is taken, on 20–25 mg of sample.

In parallel with these studies, a preliminary HPLC method is developed within ca. one week, and, after completion of minimal validation, it is used to give an estimate of the purity of the original sample, whilst IR and other spectroscopic techniques may be used to define the salt and the stoichiometry. Knowledge of the approximate purity is important at this stage, as the presence of high levels of some impurities can often hinder crystallization by 'poisoning' crystal growth or promote the formation of a specific polymorphic form. Immediately after the development of this HPLC method for use in the assessment of the main manufacturing impurities, the analytical chemists turn their attention to the development of the corresponding stability-indicating method. The development and partial validation of this method involves the use of degraded solutions of the drug substance produced in accelerated stress studies. This method takes on three weeks to complete and can then be used in the early stability evaluation of salts; their solutions and, ultimately, simple formulations.

Thus, by carefully planned studies using a small quantity of drug substance, it is possible to identify quickly the possibilities for the chemist to investigate, once larger quantities of drug substance are made. These studies may point to a small group of potential salt formers for initial evaluation; they also give the chemist ideas on possible recrystallization solvents that can be tried.

3. The Salt-Optimization Process – First Phase

3.1. Choice of the Salt Former (Counter-Ion)

The choice of salt former (counter-ion) depends on several factors. Obviously the primary factor is that it should yield a crystalline salt. Crystallinity in a salt affords a means of purification and removal of unwanted impurities. Lack of crystallinity (i.e., the salt is amorphous) normally would be expected to lead to severe problems and uncertainties, if the product intended to be developed is a solid, oral dosage form. The prospect of unexpected and uncontrolled crystallization at some stage with just one batch, creating a product recall, is something that the *Quality*, *Regulatory*, and *Marketing* groups could not live with. Absence of crystallinity is often less of a problem if the dosage form intended is a liquid.

The choice of salt is also governed largely by the acidity or basicity of the ionizable group, the safety of the counter-ion and the drug indications.

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BIO Method

Toxicological and pharmacological implications of the selected salt former must be considered, as well as the effects of the parent drug. Salt formers can be subdivided into a number of categories depending upon their functionality and purpose. Some of the most frequently used examples are listed in Table 1.

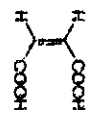
3.2. Scaling Up the Preparation of Salts

In parallel with the preliminary evaluation of opportunities for the formation of crystalline salts, the Process Chemistry group start to investigate the possibilities for economic manufacturing routes for each of the candidates remaining (usually one or perhaps two at most). The manufacturing route may

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Maleic Acid

(Z)-Butenedioic acid



pK_a

C₄H₄O₄, M_r 116.07
(1) 1.92 (A) (2) 6.23 (A)

Properties

M.p. 131 °C
Solubility (H₂O, g/100 g solution (temp. [°C])):
28.2 (0), 34.6 (10), 41.1 (20), 44.3 (25), 47.5 (30),
54.1 (40), 64.0 (50), 73.9 (80).

Safety, Toxicology

Acid: LD₅₀: mouse oral, 2400 mg/kg; rat oral,
705 mg/kg.
Disodium salt: LD₅₀: rat oral, 3380 mg/kg.
Sodium salt: LD₅₀: i.p., 600 mg/kg; TDLo: rat oral,
9 g/kg in 30 days.

In a drug-maleic study in dogs, acute tubular
necrosis could be traced back to the maleic moiety
corresponding to 9 mg/kg maleic acid (see Remarks).

Salt

Maleate

Examples

Azaxidine, brompheniramine, chlorpheniramine,
dimetindene, enalapril, levocetipromazine, levamisole,
megpromine, methylglutamine, methylerginate,
nifedipine, nonifedipine, perhexiline, pheniramine,
pivastatin, prochlorperazine, thietilperazine,
timolol, timotheine, timoprazine.

Remarks

1. May potentially isomerize to fumaric acid.
2. In a recent report [23] maleic acid could be
made responsible for acute tubular necrosis in
dogs after a single peroral dose of a test
substance supplied as a maleate (maleic acid
corresponding to a dose of 9 mg/kg
maleic acid).

(-)-L-Maleic Acid



C₄H₄O₅, M_r 134.09

Thiocyanic Acid

HS-C≡N

CHNS, M_r 59.09

pK_a

-1.33 (A)

Properties

Colorless gas or white solid, depositing upon the
degree of polymerization. Freely soluble in H₂O.

Safety, Toxicology

Ammonium salt: LD₅₀: rat oral, 750 mg/kg; mouse
oral, 500 mg/kg; i.p., 500 mg/kg; guinea pig oral,
500 mg/kg.
Sodium salt: LD₅₀: rat oral, 364 mg/kg; i.p.,
540 mg/kg; mouse oral, 362 mg/kg; i.p., 500 mg/kg;
i.v., 484 mg/kg; dog i.v., 103 mg/kg.

Salt

Thiocyanate, thiocyanide.

Examples

Dihydrocodeine

Remarks

Thiocyanate (thiocyanide, SCN⁻) is a physiological
substance, which is ubiquitously spread in the
animate nature. As an essential constituent of the
cell, it participates in important physiological and
biochemical processes [31].

para-Toluenesulfonic Acid



pK_a

-1.34 (A)

Properties

Acid: m.p. 105–107 °C, mesomorphic form m.p.
38 °C, monohydrate: m.p. 100–105 °C.
Solubility: freely in H₂O (-67 g/100 ml), soluble
in EtOH, Et₂O.

Safety, Toxicology

Acid: LD₅₀: rat oral, 2480 mg/kg; mouse oral,
400 mg/kg.
Sodium salt: LD₅₀: rat oral, 8350 mg/kg [32];
mouse i.v., 1705 mg/kg.

Salt

Tosilate (INN), mesylate (USAN).

Examples

Brevibion, bupivacaine, ibuprofen, procaine, procaine,
sulfamonomethoxyl, sulfapyridine, sulfasalazine.

1. Introduction

The preparation of pharmaceutical salts is usually not a matter of university teaching, and most of the organic chemists are not trained to prepare salts. For this reason, it appeared useful to the authors to collect some preparation methods from the literature and to present them in this chapter as possible models. Most of the 'recipes' stem from the patent literature and, therefore, are not optimized. Nevertheless they illustrate the state of the art and sometimes propose several variants for the preparation of a given salt form.

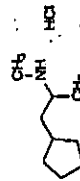
2. Preparation of Salts of Basic Drug Substances

2.1. Hydrochlorides

Hydrochlorides do sometimes require strictly anhydrous conditions to be prepared (e.g., HCl gas in anhydrous diethyl ether (Et_2O)), at other times they require stoichiometric amounts of H_2O to be present in order to yield hydrated crystals. A typical procedure is to dissolve the organic base in the minimal amount of hot isopropanol ($i\text{-PrOH}$) and to add the calculated volume of concentrated HCl (85 ml = 1 mol). If, after cooling, crystallization does not occur, it can be induced by progressive additions of Et_2O . Only if both procedures fail, salt formers other than HCl should be envisaged.

Cyclopentamine Hydrochloride *Clopaze® (Lilly)*

Procedure [1]



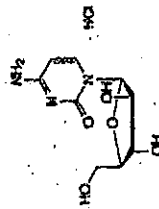
Dissolve 141 g (1 mol) of 3-cyclopentyl-*N*-methylpropan-2-amine in 500 ml of dry Et_2O , and pass dry HCl into the solution, until the weight of the mixture and container has increased by 36 g. During the addition of the HCl, the HCl salt of 3-cyclopentyl-*N*-methylpropan-2-amine precipitates as a white powder. The salt is filtered off and washed with dry Et_2O . 3-cyclopentyl-*N*-methylpropan-2-amine hydrochloride thus prepared melts at ca. 113–115 °C. The yield is practically quantitative.

Caution!

When this procedure is performed on a small scale, it is often observed that the initially formed hydrochloride re-dissolves. This is due to the fact that an excess of gaseous HCl in anhydrous Et_2O produces the very polar di-

ethyloxonium chloride, which solubilizes the initially precipitated hydrochloride.

Cytarabine Hydrochloride *Cytosar® (Upjohn)*

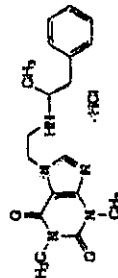


Procedure [2]

A solution of 250 mg D-1- β -arabinofuranosylcytosine in absolute methanol (MeOH) was warmed and stirred with decolorizing charcoal. The mixture was filtered through a bed of filter aid, and the filter bed was washed repeatedly with absolute MeOH. The combined filtrate and washings were pale yellow. The solution was diluted to faint cloudiness with anhydrous Et_2O , and an excess of anhydrous HCl was introduced. Crystallization began at ca. 25 °C and further crystallization was induced by chilling at 0 °C for 14 hours. The crystalline product was collected on a filter, washed with anhydrous Et_2O , and dried in air. There was thus obtained 180 mg of pale yellow D-1- β -arabinofuranosylcytosine hydrochloride melting at 186 to 189 °C.

The pale yellow product was dissolved in warm, absolute MeOH, and after treatment with decolorizing charcoal the solution was filtered through a bed of filter aid. The filter bed was washed with warm absolute MeOH, and the combined methanolic filtrate and washings were warmed and diluted with anhydrous Et_2O to incipient crystallization. The MeOH/ Et_2O mixture was kept at ca. 25 °C for ca. 1 hour and then chilled, first at 0 °C, and then at –20 °C. The resulting colorless needles were collected on a filter, washed with anhydrous Et_2O , and dried at 85 °C, yielding 100 mg of D-1- β -arabinofuranosylcytosine hydrochloride with a melting point of 186–188 °C.

Fendiline Hydrochloride *Sensit® (Tienzenun)*



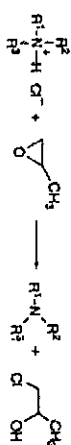
Procedure [3]

The freshly distilled base, 25.38 g of 1,7-dihydro-1,3-dimethyl-7-[(1-methyl-2-phenylethyl)amino]ethyl]-2H-pyrazolo[4,3-d]pyrimidin-2-one is dissolved in 134 ml of 96% EtOH, whereupon 26.8 ml of concentrated HCl and 201 ml of hot H_2O are added while cooling with ice-water. The precipitate is filtered off and dried in *vacuo* at 100 °C. 22.98 g of hydrochloride salt are obtained. M.p. 200–201 °C. On recrystallization from 265 ml of a 2:1 mixture of H_2O and 96% EtOH the melting point remains unchanged.

#3
#4
151

Conversion of a Hydrochloride to the Free Base

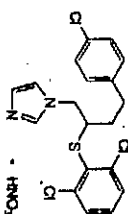
The usual way to prepare free bases from hydrochlorides and, more generally from salts, consists in adding an inorganic base (NaOH, K_2CO_3) and extracting the free base by means of a water-immiscible organic solvent. However, under some circumstances it may be desirable to work under anhydrous conditions. This can be achieved for example in bringing the hydrochloride to react with an HCl scavenger such as propylene oxide, according to the reaction below:



The 1-chloropropen-2-ol formed can be easily removed under reduced pressure. One of the interesting applications of this procedure is the transition from hydrobromides, the usual product of demethylation reactions, to the corresponding hydrochlorides, via the free base.

Procedure [4]

To a solution of 3.3 g (13.3 mmol) of L-3-hydroxy-4-methoxyphenyl-alanine hydrochloride in 150 ml of EtOH are added portionwise over 3 hours 12.5 ml propylene oxide. The mixture is allowed to stand 20 hours at ambient temperature (ca. 25 °C). The precipitated crystals are collected and purified by dissolution in 40 ml of absolute EtOH and inducing the crystallization by adding some drops of H_2O . The yield is 2.15 g of colorless crystals of L-3-hydroxy-4-methoxyphenylalanine melting at 251–252 °C.

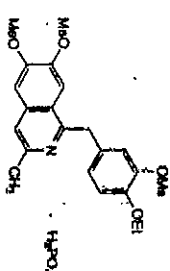
2.2. Nitrates*Butoconazole Nitrate
Femstat® (Syntex)**Procedure [5]*

To a solution of 10 g (ca. 0.025 mol) of butoconazole (= 1-(4-(4-chlorophenyl)-2-[(2,6-dichlorophenyl)sulfanyl]butyl)-1H-imidazole) base in 300 ml of dry Et_2O , 70% HNO_3 ($d=1.42$) is added dropwise until the precipitation is complete (ca. 2.2 ml). The resulting nitrate salt is recrystallized from ace-

tone/ $AcOEt$ to give butoconazole nitrate as colorless blades; yield: 9.6 g corresponding to ca. 80%.

2.3. Phosphates*Amphetamine Phosphate**Procedure [6]*

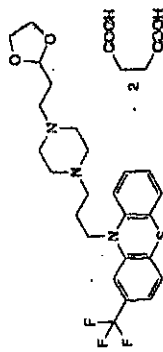
Amphetamine (= α -methylbenzenethanamine; 135 g, 1 mol) is stirred into 300 ml of acetone in a stainless-steel vessel. To the resultant solution, 115.3 g of 85% H_3PO_4 (containing 1 mol of H_3PO_4) are added under constant agitation, care being taken to avoid any sudden rise in temperature or local overheating due to the considerable amount of heat that is evolved. During the addition of the H_3PO_4 , a fine, white, flocculent precipitate appears, which becomes more and more dense and abundant, as the quantity of added acid increases. When the entire quantity of the H_3PO_4 has been added, agitation of the mixture is continued for ca. ½ hour or more to insure complete conversion. The precipitate is then allowed to settle, the supernatant liquid is discarded, and the residue is filtered. The precipitate thus separated may, if desired, be washed with acetone and is then dried by evaporation to constant weight, providing a fine, white, impalpable powder, which consists of pure monobasic amphetamine phosphate.

*Diospyne Phosphate
Paveril® (Lilly)**Procedure [7]*

A solution of 5 g of 6,7-dimethoxy-3-methyl-1-(4-ethoxy-3-methoxybenzyl)isoquinoline in 100 ml of EtOH is treated with a solution of 1.5 g of H_3PO_4 in 10 ml of EtOH. Thereafter, 10 ml of H_2O are added to effect complete solution, and the reaction mixture is then cooled, and Et_2O is added until precipitation of the salt is complete. The precipitate of 6,7-dimethoxy-3-methyl-1-(4-ethoxy-3-methoxybenzyl)isoquinoline phosphate is filtered off and recrystallized from 85% EtOH by the addition of 2 volumes of Et_2O .

2.4. Succinates

Oxaflumazine Succinate
Oxaflumine® (Diamont)

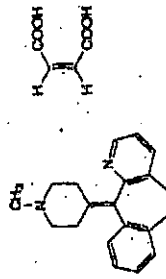


Procedure [8]

A solution of 257 g oxaflumazine (= 10-(3-(4-(2-(1,3-dioxan-2-yl)ethyl)piperazin-1-yl)propyl)-2-(trifluoromethyl)-10*H*-phenothiazine) base in 500 ml of acetone is added with stirring to a solution of 118 g of succinic acid in 4000 ml of acetone. The agitation is continued at room temperature until the desired salt crystallizes. The mixture is allowed to stand overnight at 0 °C. The weight of the collected crystals is 256 g (yield, 70%). After recrystallization in 1000 ml of acetonitrile (MeCN) the acid disuccinate melts at 136–138 °C.

2.5. Malenates

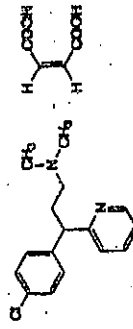
Azatidine Maleate
Optimine® (Schering)



Procedure [9]

To a solution containing 4.3 g of 6,11-dihydro-11-(1-methylpiperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine in 55 ml of AcOEt, add a solution of 3.45 g of maleic acid dissolved in AcOEt. Filter the resulting precipitate and recrystallize the desired product from an AcOEt/MeOH mixture to yield the dimaleate salt. M.p. 152–154 °C.

Dexchlorpheniramine Maleate
Polaramine® (Schering)

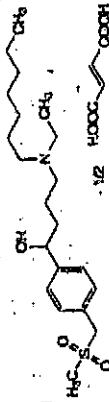


Procedure [10]

(+)-Dexchlorpheniramine (= γ -(4-chlorophenyl)-*N,N*-dimethylpyrrolidine-2-propanamine) (4.3 g) and maleic acid (1.8 g) are dissolved in 20 ml of isopropyl acetate (AcO(*i*-Pr)) and kept at room temperature until crystallization

is complete. The crystals are filtered, washed with AcOEt and recrystallized from 15 ml of this solvent in the same manner. The crystalline maleate salt so formed is filtered off and dried. M.p. 113–115 °C.

Ibutilide Fumarate
Corvert® (Pharmacia & Upjohn)

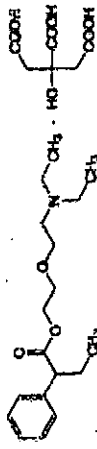


Procedure [11]

Ibutilide (= 3-[(ethyl)heptylamino]propyl)-4-[(methylsulfonyl)methyl]phenylmethanol; 31.9 g, 0.0879 mol) is dissolved in 135 ml of absolute EtOH and mixed with a solution of fumaric acid (4.3 g, 0.037 mol) in absolute EtOH (203 ml). The solution was concentrated under reduced pressure, and the residue was dissolved in hot acetone and allowed to crystallize. Recrystallization from acetone gave a solid that was dried under reduced pressure for 4 days at 60 °C to give 31.4 g of the (*E*)-but-2-enoate salt of Ibutilide (2:1). M.p. 117–119 °C.

2.6. Citrates

Buzamtrate Citrate
Sinecod® (Hommel)

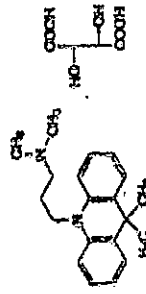


Procedure [12]

To 10 g of high-vacuum distilled 2-[2-(diethylamino)ethoxy]ethyl α -ethylbenzenesulfonate is added a solution of 7 g of citric acid in 30 ml of warm acetone. After standing for some time, the citrate crystallizes out. After suction filtration and washing with acetone, the citrate is recrystallized from acetone. M.p. 75 °C.

2.7. Tartrates

Dimetacrine Tartrate
Isonitil® (Siegfried)



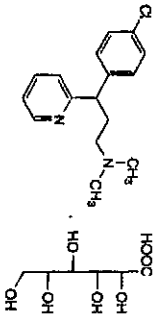
144
145
153

Procedure [13]

N,N',9,9-Tetramethyl-9H-acridine-10-propanamine (43 g) is dissolved in 229 ml of 1*N* aqueous (+)-tartaric acid, and the clear solution obtained is evaporated to dryness under reduced pressure. The residue is dissolved in 150 ml of 90% EtOH. The solution gives after cooling, the corresponding tartrate as white needles. The salt contains 1 mol of tartaric acid per mol of the base and is easily soluble in cold H₂O. M.p. 155 – 156 °C.

2.8. Gluconates

Dexchlorpheniramine Gluconate
Polaramine® (Gluconate) (Schering)

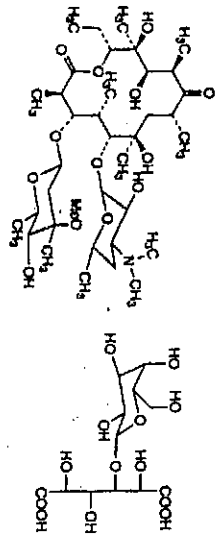


Procedure [10]

D-7-(4-Chlorophenyl)-N,N-dimethylpyridine-2-propanamine. (10 g) and 6.5 g of D-gluconolactone are mixed in 50 ml of 50% aqueous EtOH and kept at 50 °C for 2 hours. The solvent is then removed under reduced pressure to leave the desired salt as viscous colorless oil.

2.9. Lactobionates

Erythromycin Lactobionate
Erythrocin lactobionate® (Abbott)



Procedure [14]

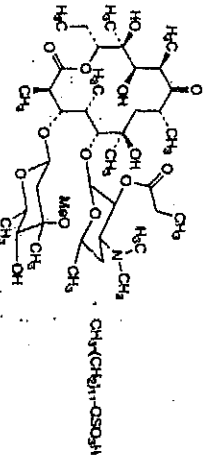
A solution of erythromycin base is prepared by dissolving 8.0 g of erythromycin in 25 ml of acetone. One the other hand, a solution of 4.0 g of δ-lactobionolactone is dissolved in 25 ml of H₂O, providing the free lactobionic acid (= 4-O-β-D-galactopyranosyl-D-gluconic acid). The two solutions are mixed and evaporated to a gummy residue, which is dissolved in 60 ml of H₂O, and

Reactions of erythromycin
Tetrahedron 1968

the resulting solution is frozen and dried *in vacuo* by lyophilization. The dried residue of erythromycin lactobionate (11.7 g) is a white amorphous powder with solubility in H₂O of ca. 200 mg/ml. M.p. 145 – 150 °C.

2.10. Lauryl Sulfate Salts

Erythromycin Estolate (Erythromycin Propionate Laurylsulfate)
Ilosone® (Disco)

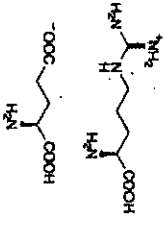


Procedure [15]

Monopropionylerythromycin (16.7 g) is dissolved in 50 ml of warm acetone. To the solution is added sodium lauryl sulfate (6.4 g), dissolved in 50 ml of distilled H₂O containing 2 ml of glacial AcOH. The white crystalline precipitate of monopropionylerythromycin lauryl sulfate separated is filtered off and dried. M.p. ca. 135 – 137 °C.

2.11. Glutamate

Arginine Glutamate
Madarnate® (Abbott)



Procedure [16]

This water-soluble salt may be prepared by mixing L-arginine with L-glutamic acid in H₂O and crystallizing the resulting salt by the addition of a polar water-miscible organic solvent. For instance, when 17.2 g of L-arginine and 14.5 g of L-glutamic acid are dissolved in 155 g of H₂O, a clear homogeneous solution results, which has a pH of 5.3. After filtration, this solution is concentrated at 50 °C under reduced pressure to a solid content of ca. 45%. Absolute MeOH (220 g) is added, and this mixture is cooled to 5 °C for 1 hour. The resulting solid salt is removed by filtration and washed with absolute MeOH. The salt is left to become air-dry and is then further dried in a vacuum oven at 60 °C for 3 hours. Thus, 30 g (94.6% of the theoretical) yield based on the amounts of

Yoshida, Unpublished
Biom. Med. Chem.



Crystalline solids

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Abstract

Many drugs exist in the crystalline solid state due to reasons of stability and ease of handling during the various stages of drug development. Crystalline solids can exist in the form of polymorphs, solvates or hydrates. Phase transitions such as polymorph interconversion, desolvation of solvate, formation of hydrate and conversion of crystalline to amorphous form may occur during various pharmaceutical processes, which may alter the dissolution rate and transport characteristics of the drug. Hence it is desirable to choose the most suitable and stable form of the drug in the initial stages of drug development. The current focus of research in the solid-state area is to understand the origins of polymorphism at the molecular level, and to predict and prepare the most stable polymorph of a drug. The recent advances in computational tools allow the prediction of possible polymorphs of the drug from its molecular structure. Sensitive analytical methods are being developed to understand the nature of polymorphism and to characterize the various crystalline forms of a drug in its dosage form. The aim of this review is to emphasize the recent advances made in the area of prediction and characterization of polymorphs and solvates, to address the current challenges faced by pharmaceutical scientists and to anticipate future developments. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Crystallinity; Polymorphs; Hydrates; Solvates; Formulation; Drug substance; Phase transformation; Characterization

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E-mail address: grant001@tc.umn.edu (D.J.W. Grant).

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1. Introduction

Most organic and inorganic compounds of pharmaceutical relevance can exist in one or more crystalline forms. When applied to solids, the adjective, *crystalline*, implies an ideal crystal in which the structural units, termed *unit cells*, are repeated regularly and indefinitely in three dimensions in space. The unit cell has a definite orientation and shape defined by the translational vectors, a , b , and c , and hence has a definite volume, V , that contains the atoms and molecules necessary for generating the crystal. Each crystal can be classified as a member of one of seven possible crystal systems or crystal classes that are defined by the relationships between the individual dimensions, a , b , and c , of the unit cell and between the individual angles, α , β , and γ of the unit cell [1,2]. The structure of a given crystal may be assigned to one of the seven crystal systems, to one of the 14 Bravais lattices, and to one of the 230 space groups [1]. All the 230 possible space groups, their symmetries, and the symmetries of their diffraction patterns are compiled in the International Tables for Crystallography [3].

The common crystalline forms found for a given drug substance are polymorphs and solvates. Crystalline polymorphs have the same chemical composition but different internal crystal structures and, therefore, possess different physico-chemical properties. The different crystal structures in polymorphs arise when the drug substance crystallizes in different crystal packing arrangements and/or different conformations. The occurrence of polymorphism is quite common among organic molecules, and a large number of polymorphic drug compounds have been noted and catalogued [4–7].

Solvates, also known as pseudopolymorphs, are

crystalline solid adducts containing solvent molecules within the crystal structure, in either stoichiometric or nonstoichiometric proportions, giving rise to unique differences in the physical and pharmaceutical properties of the drug. If the incorporated solvent is water, a solvate is termed a hydrate. Adducts frequently crystallize more easily because two molecules often can pack together with less difficulty than single molecules. While no definite explanations can be given, possible reasons include adduct symmetry, adduct-induced conformation changes, and the ability to form hydrogen bonds through the solvent molecules [2,8,9]. Desolvated solvates are produced when a solvate is desolvated and the crystal retains the structure of the solvate [10]. Desolvated solvates are less ordered than their crystalline counterparts and are difficult to characterize, because analytical studies indicate that they are unsolvated materials (or anhydrous crystal forms) when, in fact, they have the structure of the solvated crystal form from which they were derived [11].

Because different crystalline polymorphs and solvates differ in crystal packing, and/or molecular conformation as well as in lattice energy and entropy, there are usually significant differences in their physical properties, such as density, hardness, tableability, refractive index, melting point, enthalpy of fusion, vapor pressure, solubility, dissolution rate, other thermodynamic and kinetic properties and even color [12]. Differences in physical properties of various solid forms have an important effect on the processing of drug substances into drug products [13], while differences in solubility may have implications on the absorption of the active drug from its dosage form [14], by affecting the dissolution rate and possibly the mass transport of the molecules. These concerns have led to an increased regulatory

interest in understanding the solid-state properties and behavior of drug substances. For approval of a new drug, the drug substance guideline of the US Food and Drug Administration (FDA) states that “appropriate” analytical procedures need to be used to detect polymorphs, hydrates and amorphous forms of the drug substance and also stresses the importance of controlling the crystal form of the drug substance during the various stages of product development [11]. It is very important to control the crystal form of the drug during the various stages of drug development, because any phase change due to polymorph interconversions, desolvation of solvates, formation of hydrates and change in the degree of crystallinity can alter the bioavailability of the drug. When going through a phase transition, a solid drug may undergo a change in its thermodynamic properties, with consequent changes in its dissolution and transport characteristics [15].

Various pharmaceutical processes during drug development significantly influence the final crystalline form of the drug in the dosage form. The various effects of pharmaceutical processing on drug polymorphs, solvates and phase transitions have been described in detail by Brittain and Fieser [16] and will be discussed in later chapters. Briefly, processes such as lyophilization and spray drying may lead to the formation of the amorphous form of drug, which tends to be less stable and more hygroscopic than the crystalline product. Also, processing stresses, such as drying, grinding, milling, wet granulation, oven drying and compaction, are reported to accelerate the phase transitions in pharmaceutical solids. The degree of polymorphic conversion will depend on the relative stability of the phases in question, and on the type and degree of mechanical processing applied. Keeping these factors in mind, it is desirable and usual to choose the most stable polymorphic form of the drug in the beginning and to control the crystal form and the distributions in size and shape of the drug crystals during the entire process of development. The presence of a metastable form during processing or in the final dosage form often leads to instability of drug release as a result of phase transformation [17].

Crystallization plays a critical role in controlling the crystalline form and the distribution in size and shape of the drug. The significance of crystallization

mechanisms and kinetics in directing crystallization pathways of pharmaceutical solids and the factors affecting the formation of crystals have been reviewed in detail by various researchers [12,18,19]. A crystalline phase is created as a consequence of molecular aggregation processes in solution that lead to the formation of nuclei, which achieve a certain size during the nucleation phase to enable growth into macroscopic crystals to take place during the growth phase. The factors affecting the rate and mechanisms by which crystals are formed are: solubility, supersaturation, rate at which supersaturation and desupersaturation occur, diffusivity, temperature, and the reactivity of surfaces towards nucleation. The various forces responsible for holding the organic crystalline solids together, such as nonbonded interactions and hydrogen bonding, have been discussed in detail by Byrn et al. [2] and Etter [20].

Various analytical methods are being currently used to characterize the crystalline form of the drug during the various steps of processing and development. These methods have been reviewed recently in detail by many authors [7,10,21–25]. The single most valuable piece of information about the crystalline solid, including the existence of polymorphs and solvates, is the molecular and crystalline structure, which is determined by single-crystal X-ray diffractometry [2]. Powder X-ray diffractometry provides a “fingerprint” of the solid phase and may sometimes be used to determine crystal structure. Once the existence of polymorphism (or solvate formation) is definitely established by single-crystal and powder X-ray diffractometry, spectral methods, such as Fourier transform infrared absorption (FTIR) spectroscopy, Fourier transform Raman scattering (FT Raman) spectroscopy, solid-state nuclear magnetic resonance (SSNMR) spectroscopy, ultraviolet and visible (UV-Vis) and/or fluorescence spectroscopy [23] may be employed for further characterization. Of special significance are thermal methods, such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and optical microscopy using a hot stage [24]. These methods are almost always employed for further characterization. Modulated (temperature) differential scanning calorimetry (MDSC) in combination with DSC and optical microscopy are able to identify the glass

transition of amorphous forms with much greater clarity and allow unique insights into the glass transitional and polymorphic behavior of drug substances [26].

Because solid-state NMR spectroscopy can be used to study crystalline solids, as well as pharmaceutical dosage forms, this powerful method is finding increasing application in deducing the nature of polymorphic variations [27], such as variations in hydrogen bonding network and molecular conformations among polymorphs [28,29] and for the determination of molecular conformations and mobility of drugs in mixtures and dosage forms [2]. Solid-state ^{13}C -NMR in conjunction with the techniques, known as high power proton decoupling, cross polarization (CP), and magic-angle spinning (MAS) offers information not obtained readily by other techniques. Recently, two-dimensional ^{13}C -solid-state NMR spectroscopy has been used to study the three conformational polymorphs of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile [30]. Use of two-dimensional NMR and total suppression of spinning side bands (TOSS) pulse sequences allowed the separation of isotropic and anisotropic chemical shifts for the three forms. This is a very powerful method for analyzing differences in the chemical environment and is finding increased application in the study of conformational polymorphism.

With advances in analytical methods, the current focus of research in the solid-state area is to understand polymorphism and pseudopolymorphism at the molecular level. Knowledge of the crystal packing arrangements and the various intermolecular forces involved in the different packing arrangements will help in the prediction and preparation of the most stable polymorphs of a given compound well in advance, to avoid surprises during product development. A current emphasis is on the development of software to predict crystal structures of polymorphs from molecular structures. A thorough understanding of the physicochemical properties of polymorphs and solvates (hydrates) is of primary importance to the selection of a suitable crystalline form and development of a successful pharmaceutical product. Bray et al. [31] have shown that, by thorough characterization of four different crystalline forms of L-738,167, a fibrinogen receptor antagonist by various analytical

techniques, it was possible to determine the suitability of one or two forms for the development of pharmaceutical oral dosage forms.

The present review aims to emphasize the recent advances made in the area of prediction and characterization of polymorphs and solvates, attempts to address the current challenges and problems faced by pharmaceutical scientists and intends to anticipate future development. This review does not attempt to provide solutions to the problems but attempts to review comprehensively the advances made in recent years to help address these problems.

2. Recent advances in the identification, prediction and characterization of polymorphs

2.1. Types of polymorphism

Based on differences in the thermodynamic properties, polymorphs are classified as either enantiotropes or monotropes, depending upon whether one form can transform reversibly to another or not. In an enantiotropic system, a reversible transition between polymorphs is possible at a definite transition temperature below the melting point. In a monotropic system, no reversible transition is observed between the polymorphs below the melting point. Four useful rules have been developed by Burger and Ramburger [32,33] to determine qualitatively the enantiotropic or monotropic nature of the relationship between polymorphs. These rules are the heat of transition rule, heat of fusion rule, infrared rule and density rule.

If, by use of the above rules, it is established that the polymorphs of a particular drug are enantiotropic or monotropic, then the next goal is to define the thermodynamically stable (or metastable) domain of each crystalline phase of a substance as a function of temperature. The plot of the Gibbs free energy difference, ΔG , against the absolute temperature, T , gives the most complete and quantitative information on the stability relationship of polymorphs [22], with the most stable polymorph having the lowest Gibbs free energy. The ΔG between the polymorphs may be obtained using several techniques operating at

different temperatures, such as solubility [34] and intrinsic dissolution rate. Yu [35] has derived thermodynamic equations to calculate ΔG between two polymorphs and its temperature slope from the melting data. This method is essentially an extension of the heat of fusion rule, which is based on statistical mechanics. Extrapolating ΔG to zero gives an estimate of the transition temperature, from which the existence of monotropy or enantiotropy is inferred. The integration of different types of data provides the ΔG vs. T curve over a wide temperature range and allows the consistency between techniques to be checked [22]. Another approach to establish the order of stability among various polymorphs has been studied using pressure versus temperature plots, e.g., for sulfanilamide and piracetam [36]. This approach is based upon Ostwald's principle of least vapor pressure, according to which the stable polymorph exhibits the lowest vapor pressure. The accuracy of this approach to establish the stability hierarchy among the polymorphs has been shown to be very much dependent on the accuracy of the experimental data.

In recent years, the main focus of research has been the characterization of polymorphs arising from structural differences in the crystal lattice. It has been established for some time that organic molecules are capable of forming different crystal lattices through two different mechanisms. One of the mechanisms is termed *packing polymorphism*, and represents instances where conformationally relatively rigid molecules can be assembled into different three-dimensional structures through the invocation of different intermolecular mechanisms. The other mechanism is termed *conformational polymorphism* and arises when a nonconformationally rigid molecule can be folded into different arrangements, which subsequently can be packed into alternative crystal structures. The distinction between *packing polymorphism* and *conformational polymorphism* is somewhat artificial because different packing arrangements impose different conformations on the molecules, however slight, and different conformations will inevitably pack differently. The structural aspects associated with polymorphs have been reviewed recently [2], as have the analogous features of solvate and hydrate systems [9]. In the next

section, the results of some more recent investigations are discussed.

2.2. Packing polymorphism

An investigation into the structures and charge densities of two polymorphs of *p*-nitrophenol has been performed with the aim of deducing the different modes of inter-molecular hydrogen bonding that lead to the formation of the two structures shown in Fig. 1a and b [37]. A detailed analysis of the charge density of the two forms indicates charge migration from the benzene ring region to the nitro and hydroxyl groups that accompanies the transformation of one form into the other. In addition, polarization of the oxygen lone-pair electrons was found to be substantially larger in the crystal forms than in the free molecule, resulting in considerably larger dipole moments in the solid state.

During the study of a new crystal form (form I) of

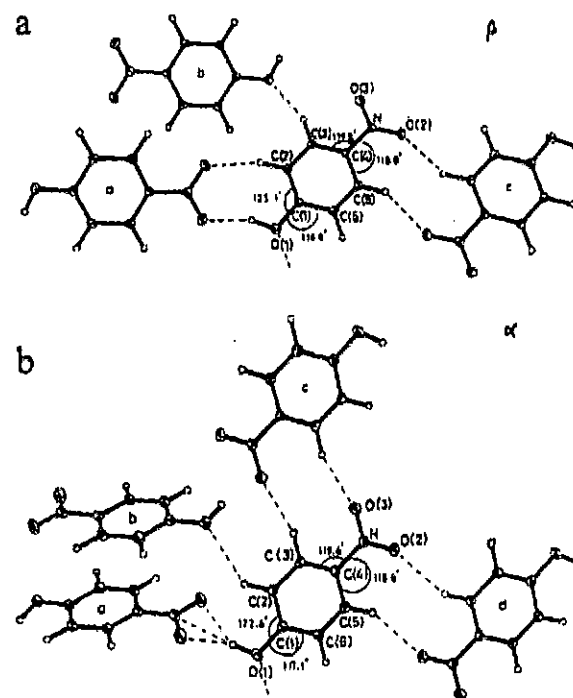


Fig. 1. Molecular packing diagrams of the (a) β polymorph of *p*-nitrophenol, (b) α polymorph of *p*-nitrophenol, showing 50% probability displacement ellipsoids ([37], reproduced with the permission of the American Chemical Society).

chlordiazepoxide, it was found that the heat of transition between the two forms (forms I and II) is rather modest, and kinetic factors permit the existence of the metastable phase [38]. Both structures contain four crystallographically independent molecules linked in dimers through hydrogen bonding, but the dimers are packed differently to yield the two crystal forms. Because the dimers in the fundamental units are spaced differently in the two forms, it was proposed that the solid-state enantiotropic transformation entailed rearrangement of the dimer units.

A different approach has been taken during an evaluation of the different structures formed by sulfathiazole [39]. Using a graph set approach to classify the known structural differences and similarities among the various forms, it became possible to identify packing motifs common to three of the four crystal structures. Fig. 2 shows the unit cells of the polymorphs I, II, III and IV, where molecules are paired as hydrogen-bonded dimers. At the end of the process, the authors were able to deduce possible links between the observed patterns of hydrogen bonding, processes of nucleation, and the crystal growth observed from a number of solvent systems. Interestingly, the analysis did not indicate a relationship between the appearance of a particular polymorph from solution and the growth of its fastest

growing surface. Rather, it appeared as if the different solvents affected the process of polymorph formation through their effects on nucleation of the various forms.

2.3. Conformational polymorphism

The conformational polymorphism of the two forms of piroxicam pivalate has been studied in detail [40]. This compound is distinctive in that the high-melting form (polymorph 1) contains an unanticipated array of associated molecules bound as centrosymmetric dimers through hydrogen bonding, with the amido nitrogen atom acting as the donor and the pyridine nitrogen as the acceptor (Scheme 1, structure I). The low-melting form (polymorph 2) contains molecules of two distinct conformational states coexisting in the same crystal (Fig. 3), but linked through different hydrogen bonding arrangements. This latter finding represents another unusual aspect of the crystallography of the substance.

The inclusion of different solvent molecules in a crystal lattice can lead to the existence of different packing patterns, and has also been found to influence the molecular conformation of paroxetine hydrochloride in two solvate forms [41]. One form

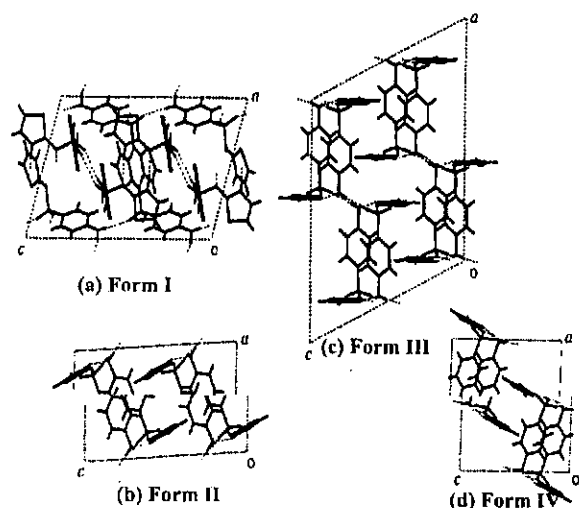
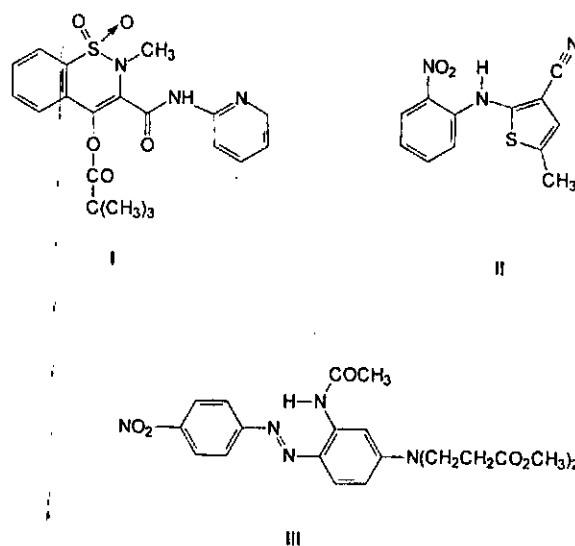


Fig. 2. Unit cells of four polymorphs (I, II, III and IV) of sulfathiazole showing hydrogen bonds, with the dimer structure clearly discernible ([39], reproduced with the permission of the Royal Society of Chemistry).



Scheme 1. Molecular structure of piroxicam pivalate (I) [40], 5-methyl-2-[2-(nitrophenyl)amino]-3-thiophenecarbonitrile (II) [30], 2'-acetamido-4'-[N,N-bis(2-methylcarbonyl)ethyl]amino]-4-nitroazobenzene (III) [48].

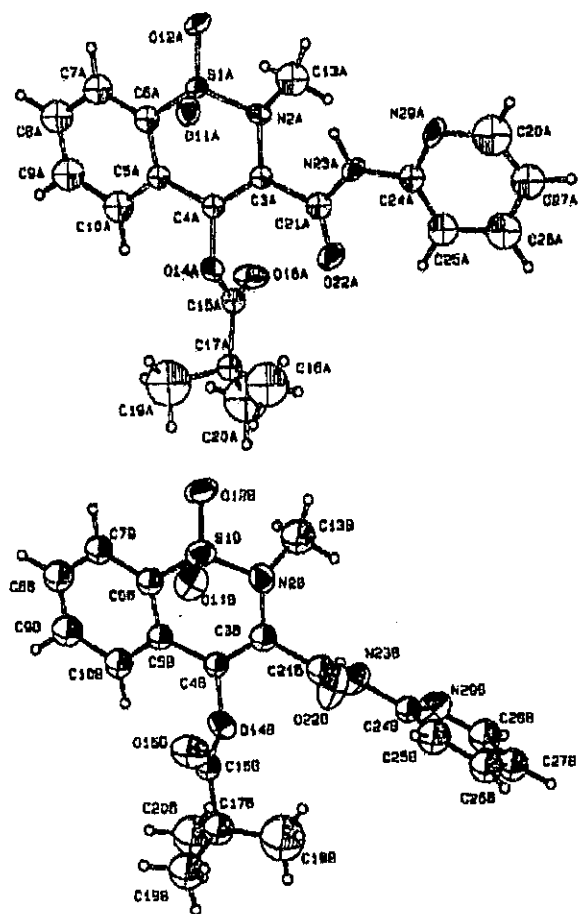


Fig. 3. Conformations of the two independent molecules of piroxicam pivalate (I) in polymorph 2. Thermal ellipsoids are drawn at the 40% probability level, and H atoms are shown as spheres of arbitrary size ([40], reproduced with the permission of the American Pharmaceutical Association).

was obtained as a hemihydrate, and the other as the solvate of isopropanol (2-propanol). In the unit cell of the hemihydrate, one finds two protonated paroxetine and two chloride ions together with one water molecule. Interestingly, the two paroxetine molecules are conformationally nonequivalent, and exhibit a number of different bond angles and torsion angles. In the other form, the unit cell contains one protonated paroxetine molecule, one chloride ion, and one isopropanol molecule disordered along a molecular channel. Furthermore, the conformation of the paroxetine molecule in the isopropanol solvate is different from either molecular conformation observed in the

hemihydrate phase. Crystals of the isopropanol solvate decomposes in the open air at room temperature, because the isopropanol molecules are released easily through the channel. The hemihydrate is relatively stable.

In an impressive fundamental study, the polymorphism of 5-methyl-2-[2-(nitrophenyl)amino]-3-thiophenecarbonitrile (Scheme 1, structure II) has been catalogued [42,43] and discussed in detail [2]. This compound was crystallized as six solvent-free polymorphs, each of which differed in the mode of packing and in molecular conformation. The different conformers yielded sufficient perturbations on the respective molecular orbital so that a variety of crystal colors (red, orange, and yellow) were observed. To obtain a more detailed evaluation of the relative stability, the authors considered a partitioning of polymorphic energy differences into lattice and conformational contributions, and were able to deduce general trends that appeared valid in the absence of hydrogen bonding. The act of crystallization was found to feature an interplay of opposing forces, with perpendicular molecular conformations being favored in fluid solutions, while a preference for planar/high dipole conformers existed in most crystal forms, as shown in Fig. 4 [42]. The unusual polymorphism displayed by this system may result from one or more of the following factors: the preference for perpendicular conformations in solutions, the preference for planar/high dipole conformers in crystals, the formation of inter- and intramolecular hydrogen bonds, and the thermodynamic tendency towards low energy and high entropy.

2.4. Phase transformations in the solid state

Studies of phase transformations in the solid state are important, because the sudden appearance or disappearance of a crystalline form can threaten process development, and can lead to serious pharmaceutical consequences if the transformation occurs in the dosage forms. Hence, an understanding of the kinetics and mechanism of phase transformations is of practical importance. The rearrangement of molecules into a new structure during phase transformation may or may not involve a solvent or vapor phase. To explain the mechanism of solid–solid

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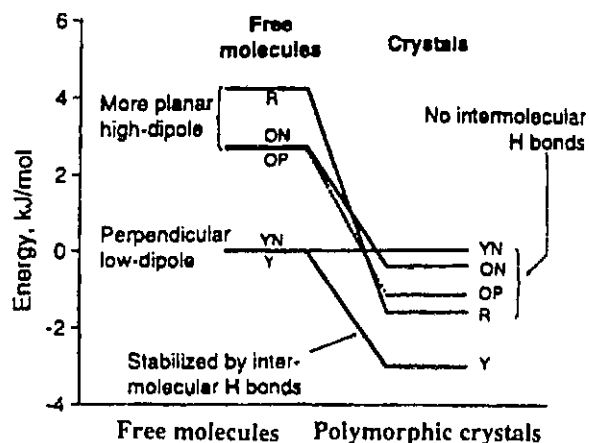


Fig. 4. Comparison of conformational energies and crystal energies of the various polymorphs of 5-methyl-2-[2-(nitro-phenyl)amino]-3-thiophenecarbonitrile (II). Form R (red prisms, m.p. 106.2°C), form Y (yellow prisms, m.p. 109.8°C), form OP (orange plates, m.p. 112.7°C), form ON (orange needles, m.p. 114.8°C), form YN (yellow needles, m.p. not measurable) ([42], reproduced with the permission of the American Chemical Society).

physical transition, four steps have been proposed: (a) molecular loosening in the initial phase; (b) formation of an intermediate solid solution; (c) nucleation of the new solid phase and (d) growth of the new phase [2]. In an interesting study, Skwierczynski [44] has proposed a two-environment model to describe the decomposition reaction kinetics of a crystalline solid, aspartame. The decomposition reaction of aspartame is a simple unimolecular thermally-induced aminolysis and the reaction proceeds under anhydrous conditions, i.e., water is not a reactant [45]. This model links the chemistry of the solid-state reaction with the molecular mobility of the reactant as the reaction proceeds. The advantage of this model is that it can be used to determine the shelf life of a product from kinetic data gathered at elevated temperatures. Apart from solid–solid physical transformations, solution-mediated physical transformations among polymorphs are also known to occur in processes, such as wet granulation and during dissolution testing.

While the majority of studies have probed the equilibrium properties of polymorphic and solid-state solvated systems, relatively few have been concerned with the dynamics of phase transformation. Byrn et

al. [2] have reviewed briefly the aspect of polymorphic interconversions and the factors affecting the transformations. In one study, the contribution of hydrogen bonding to the $\alpha \rightarrow \beta$ phase change of resorcinol has been detailed [46]. The α form is more stable than the β form at room temperature, but is less dense than the β form. The transition of $\alpha \rightarrow \beta$ at an estimated transition temperature of 337 ± 1 K is accompanied by an increase in crystal density, with the structure shifting from an open array of molecules (linked through hydrogen bonding) to a denser structure resembling molecular crystals. Through the use of a simple potential model, it was concluded that, during the phase transformation, the energy of the hydrogen bonds decreases along with the extent of such bonding. The energy liberated by this process is almost offset by the enhanced Van der Waals energies associated with the increase in crystal density, and consequently the transition enthalpy is rather small. Accompanying the shifts in hydrogen bonding is a number of effective proton transfers, altering the covalent and ionic portions of the crystal. It was also learned that the increase in entropy produced from the redistribution of protons was of the same order of magnitude as the entropy of the phase transition.

A number of spectroscopic techniques have been used to study the processes associated with a polymorphic transition of 2-(2,4-dinitrobenzyl)-3-methylpyridine [47]. The two interconverting structures coexisted over a temperature range of at least 8–9°C. The phase change was associated with a molecular tautomerization that translated through the collective changes of a large number of molecules, yielding domains having definite short-range order. The slowly evolving spectroscopy that took place above the transition temperature was interpreted as the annealing of domains into a long-range ordered system. The process of phase transformation appeared to consist of an initial fast redistribution of the mole ratio of the coexisting phases, followed by a much slower process involving a macroscopic relaxation of the system. Although local thermodynamic equilibrium was thought to exist in individual domains, the magnitude noted for the temperature range of the phase transition was proposed to arise from nonequilibrium conditions existing among the various types of domain.

A combination of solid-state ^{15}N -NMR spectroscopy and X-ray crystallography was used to study polymorphic transitions in an azobenzene dyestuff, 2'-acetamido-4'-[*N,N*-bis(2-methoxycarbonylethyl)amino]-4-nitroazobenzene (Scheme 1, structure III) [48]. This work established that the structure of one polymorph was disordered, and that the process of phase transformation entailed a crankshaft-type motion of the azo linkage. The ORTEP plots of the two molecular conformations for the X-ray structure determination of structure III at 293 K are shown in Fig. 5. Selective polarization inversion and band shape-fitting experiments were used to deduce the thermodynamic parameters of the exchange process.

Raman spectroscopy was used to study the effect of pressure on the phase transitions in hexamethylbenzene and hexa(methyl- d_3)benzene [49]. The form II $\rightarrow$ form III transition of the partially deuterated substance was found to take place at a lower pressure relative to that of the analogous hexamethylbenzene compound, which was attributed to differences in the energies of the intramolecular methyl torsional vibration in the two crystal forms. In another study performed by the same group, the effects of both temperature and pressure on the phase transitions of tetrafluoro-1,4-benzoquinone were considered [50]. In this system, the changes in en-

vironmental conditions were found to influence a number of intermolecular and intramolecular vibrational modes, yielding conformational changes that in turn produced the observed phase transitions.

2.5. Prediction of polymorphs

The main challenge in managing the phenomenon of multiple solid forms of a drug is the inability to predict the number of forms that can be expected in a given case. This prediction would involve quantification of the myriad intermolecular forces within any proposed crystal structure as well as the ability to postulate the likely packing modes for a given molecule in all its configurations [10]. Accurate theoretical prediction of polymorphs from studies of molecular dynamics and crystal structure generation would be of outstanding importance in drug research [36].

More research is now being directed towards developing computational tools to understand the nature of polymorphism and to predict polymorphic forms at an early stage in the drug development process. The recent developments in computational chemistry allow the prediction of possible polymorphic forms based only on the molecular structure of the drug. The Polymorph Predictor, from Molecular Simulations, is currently the only commercial software package that can predict the possible polymorphs of an organic compound from its molecular structure [51]. The package developed by Karfunkel and co-workers [52–54] uses a Monte Carlo simulated annealing approach to generate thousands of possible crystal packing alternatives for a given molecule. Each of the unique crystal structures is then subjected to a lattice energy minimization to obtain the relative stability ranking of the various packing possibilities and the resulting lowest-energy structures are the potential polymorphs. This method has been successfully employed to generate known polymorphs of primidone (Fig. 6A and B) and progesterone, starting from the molecular structures alone [55]. It has also been used to predict polymorphs for a range of small molecules and to predict unknown polymorphic structures of 4-amidinolindanone guanylylhydrazone, a selective inhibitor of *S*-adenosylmethionine decarboxylase [56], and of aspirin [57].

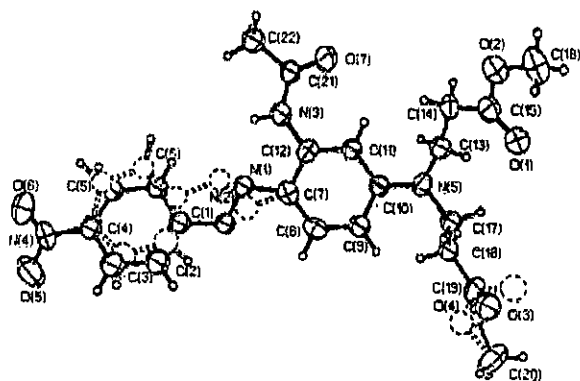


Fig. 5. ORTEP plots of the two molecular conformations (1 and 2) for the X-ray structure determination of 2'-acetamido-4'-[*N,N*-bis(2-methoxycarbonylethyl)amino]-4-nitroazobenzene (Scheme 1 Structure) at 293 K. Thermal ellipsoids are shown at 30% for clarity, with conformer 2 being represented by the solid lines and Conformer 1 by the dotted lines ([48], reproduced with the permission of the American Chemical Society).

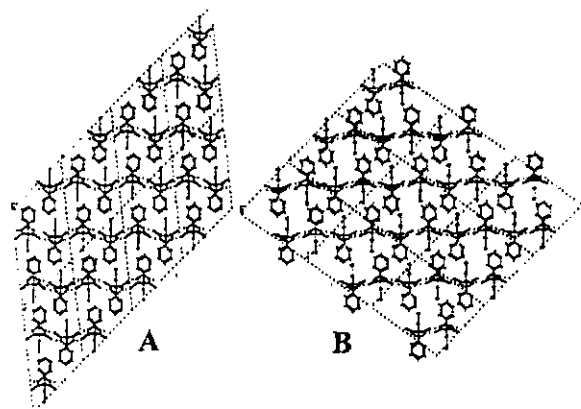


Fig. 6. Comparison of the crystal structure of primidone A (A) versus the most likely packing arrangement, frame 7 (B) ([55], reproduced with the permission of Elsevier Science).

The theoretical predictions of lattice energies, entropies, morphologies and polymorphs should stimulate experimental activities and vice versa. The current crystal-modeling efforts have the potential of producing more quantitative tools for bridging structures and properties, which could help in creating solid forms with desired properties [22]. There are many limitations in using computational methods for predicting polymorphs theoretically. The first limitation is that the *ab initio* screening is useful only for nonionic rigid molecules. For more complex systems, the method is very useful for generating plausible crystal structures, but it is not accurate enough to determine which of these possible structures can actually be crystallized [58]. In addition, the limitations in computer power can restrict the use of this method for predicting polymorphs of complex molecules. An issue of concern is that the existing methods only predict the lattice energies, which relate to internal energies or enthalpies of the crystals. However, the relative thermodynamic stability of polymorphs is determined by the Gibbs free energy, which is a linear function of both enthalpy and entropy. Predictions of the relative stability of polymorphs will be more accurate when the entropies, as well as lattice energies, are considered. Application of molecular dynamics may enable the entropies to be calculated. Hence, no general method is currently available for the prediction or interpretation of the properties of complicated polymorphic or pseudopolymorphic systems.

2.6. Directing the crystallization of specific polymorphs

Complementing the different computational methods for predicting the stable polymorphs of a given compound, various experimental methods are also being employed extensively to control the type of polymorph formed during the crystallization process. Many studies have reported the role of additives in controlling the outcome of the crystallization process. Some of the preselected additives are capable of inhibiting the nucleation and/or growth of the unwanted polymorphs. For the first time, the role of reaction by-products in controlling polymorph appearance of a drug has been reported [59]. This drug is sulfathiazole that is known to exist as polymorphs, forms I, II, III and IV, that differ in the hydrogen-bond network. Form I was found to be different from the other three forms as a result of a different hydrogen bonding at the aniline moiety of the molecule. From studies of the hydrogen-bonding pattern, it was predicted that the ethamido derivative of sulfathiazole could selectively control the formation of form I over other forms by entering the growing face of form I without disrupting the structure (Fig. 7a). Because a similar effect was not possible with the other forms, incorporation of the ethamido derivative in the other forms should inhibit their growth (Fig. 7b). Experimentally, it was shown that the ethamido by-product stabilized form I over the other polymorphs. This study clearly shows that the combination of crystal morphology and the hydrogen-bond network analysis of the different polymorphs offer a new and powerful approach to understanding and controlling polymorph appearance and stability in the presence of additives.

A similar approach was also applied to stabilize a metastable α conformational polymorph of L-glutamic acid using additives [60]. Methods such as DREIDING and TRIPOS force fields were used to select appropriate additives which could mimic the α and β conformations. Four additives were chosen for this study of which two were present exclusively in the β conformation and theoretically should selectively inhibit the crystallization of the β phase and thus stabilize the metastable α phase. Experimentally, it was proven that the additives, by virtue of their conformation, were able to selectively inhibit the

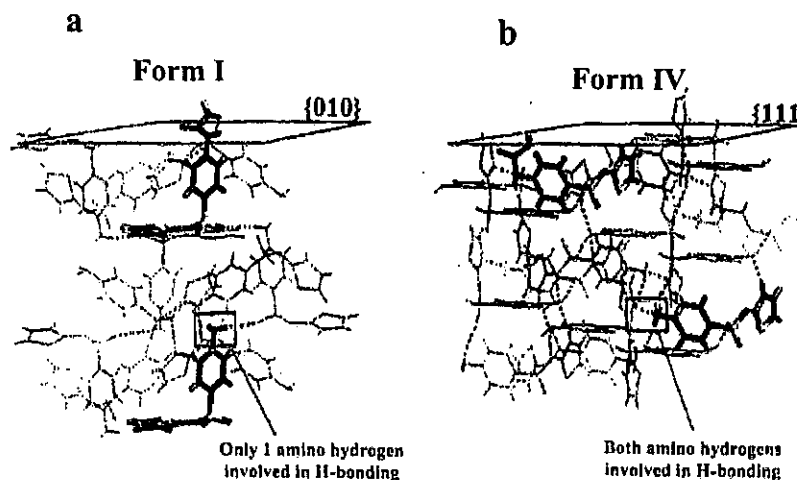


Fig. 7. Possible binding interaction of ethamidosulfamide in the fastest growing faces of (a) form I and (b) form IV of sulfathiazole ([59], reproduced with the permission of Elsevier Science).

appearance of the stable β polymorph of L-glutamic acid by interfering with either the nucleation rates or the growth rates and thus stabilize the metastable form. These studies demonstrate clearly that the molecular packing and intermolecular hydrogen bonds are the main features, which make possible the conformational discrimination. The use of conformational mimicry to stabilize the metastable structures of conformational polymorphs now offers a powerful tool for the prediction and development of robust processes for the control of polymorphic systems.

2.7. Characterization of polymorphs using a combination of analytical techniques

The common techniques often fail to differentiate definitively between two structurally similar polymorphs. Hence more advanced techniques or a combination of techniques need to be used to avoid errors of interpretation and in the identification of polymorphs [24]. Combinations of techniques are being employed currently for the characterization of crystalline pharmaceutical solids. For example, conventional single-crystal X-ray diffractometry and polarized microscopy were of no use in distinguishing the two forms I and II of roxifiban, a very promising cardiovascular drug, because of the relatively small crystallite sizes of the polymorphs. Hence, transmission electron microscopy (TEM) and

synchrotron X-ray diffraction techniques were employed to characterize the unit cells of the two forms. By coupling the highly resolved synchrotron powder X-ray diffraction data shown in Fig. 8, with in-

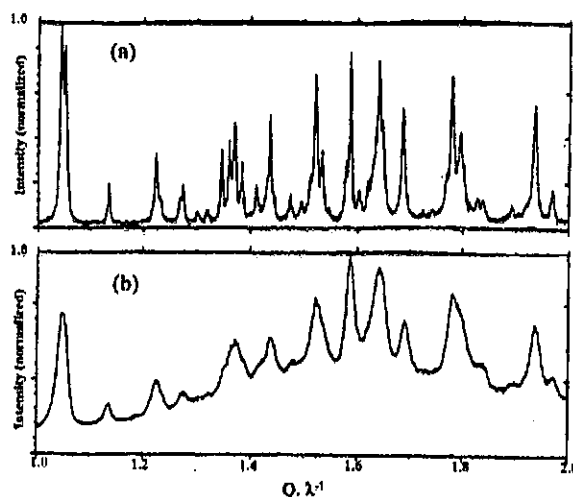


Fig. 8. A partial comparison of (a) a synchrotron pattern of polymorph II of roxifiban, collected using a wavelength of 1.00006 Å with (b) a conventional X-ray diffraction pattern using CuK α radiation in a region where there are many overlapping peaks. The patterns are plotted as a function of $Q = 2\pi/d = 4\pi \sin \theta/\lambda$ to remove the effects of different wavelengths ([61], reproduced with the permission of the American Pharmaceutical Association).

formation obtained from TEM diffraction patterns, the unit cell parameters of the two forms of roxifiban were determined [61]. Similarly, the three modifications, I, II and III, of the nonsteroidal antiinflammatory drug, tiaprofenic acid, could not be distinguished by the two traditional spectroscopic methods, FTIR and FT-Raman spectroscopy. The modifications can only be distinguished by a combination of thermoanalytical and powder X-ray diffractometric methods [62].

Another example, to which a combination of techniques has been successfully applied to identify the various conformational polymorphs of a drug, is the characterization of the solid forms of neotame [29]. Neotame, *N*-(3,3-dimethylbutyl)-L-aspartyl-L-phenylalanine methyl ester, a new high-potency sweetener exists in the following phase-pure crystalline forms: monohydrate, the most stable crystalline form of neotame under ambient conditions, a methanol + water solvate [63], a methanol solvate [64], an amorphous anhydrate [29] and a crystalline anhydrate (form A; [65]). The authors conducted a systematic study of the conversion of the monohydrate under vacuum to a mixture of anhydrate forms followed by the reconversion of the anhydrate to the monohydrate upon exposure to moisture under ambient conditions. No significant changes were observed in the powder X-ray diffraction patterns during part of the reconversion process, suggesting that no change in lattice structure had occurred. However, the solid-state ^{13}C -CP-MAS NMR spectra, indicated the presence of several forms of neotame during the reconversion (Fig. 9). This discrepancy in the results between the two techniques was attributed to the conformational change of neotame molecules during reconversion, without significant change in unit cell parameters. This example indicates that both solid-state ^{13}C -CP-MAS NMR spectroscopy and powder X-ray diffractometry are needed to analyze mixtures of solid forms of conformationally flexible molecules, such as neotame.

A combination of solid-state ^{13}C -NMR spectroscopy and single crystal X-ray diffractometry also has been used to examine the solid-state tautomerism of acetohexamide [66,67]. Polymorphism of the anti-diabetic drug acetohexamide has been investigated by numerous techniques. On the basis of FTIR data, form A of acetohexamide has been proposed to exist

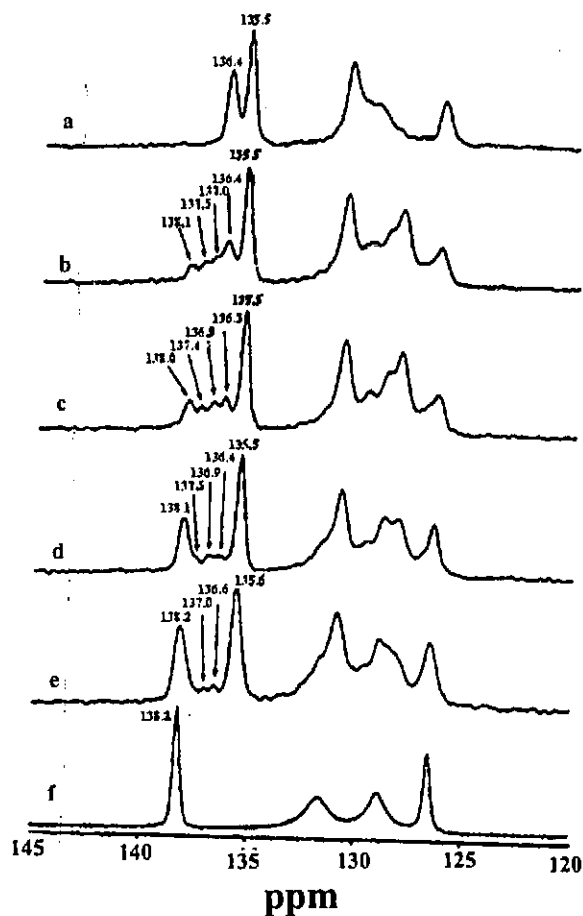


Fig. 9. Resonance signal of the phenyl carbon (C-15) attached to the side chain in the ^{13}C -CP-MAS NMR spectra of neotame anhydrate: (a) sample generated by placing the original monohydrate under vacuum (~ 1 Torr) for 3 days; (b–e) sample after being sealed in a jar for 2, 4, 6 and 8 days, respectively; (f) sample after being exposed to a relative humidity environment of 84% for 12 days ([29], reproduced with the permission of the American Chemical Society).

in the enol-tautomeric state, whereas form B has been proposed to be in the keto-tautomeric state. Using NMR and crystal structure data it was firmly established that both these acetohexamide polymorphic forms are present in the keto-form. Hence the combination of solid-state NMR spectroscopy and X-ray crystallography provided strong evidence that both forms of acetohexamide exist in the keto-tautomeric state and are truly polymorphic.

3. Recent advances in the identification and characterization of hydrates and solvates

3.1. Introduction to solvates and hydrates

It has been estimated that approximately one-third of the pharmaceutically active substances are capable of forming crystalline hydrates [68]. The water molecule, because of its small size, can easily fill structural voids and because of its multidirectional hydrogen bonding capability, is also ideal for linking a majority of drug molecules into stable crystal structures [2]. The mere presence of water in a system is not a sufficient reason to expect hydrate formation, because some compounds, though they are soluble in water, do not form hydrates. It is the activity of water in the medium that determines whether a given hydrate structure will form. Solvates may be formed when a pure organic solvent or a mixture of solvents is used as the solvent for crystallizing the compound. Guillory [69] has discussed the various methods of preparation of hydrates and solvates in detail. Because solvates behave similarly to hydrates, common analytical techniques can be used for characterization of solvates and hydrates.

3.2. Structural aspects

Crystalline hydrates, based on their structure may be classified into three categories. The first category (class I) are the isolated site hydrates, where the water molecules are isolated from direct contact with other water molecules by intervening drug molecules, e.g., cephadrine dihydrate. The second category (class 2) are channel hydrates where the water molecules included in the lattice lie next to other water molecules of adjoining unit cells along an axis of the lattice, forming channels through the crystal, e.g., ampicillin trihydrate. The channel hydrates can be subclassified into two subcategories. One category comprises the expanded-channel or nonstoichiometric hydrates, which may take up additional moisture in the channels when exposed to high humidity and for which the crystal lattice may expand or contract as the hydration or dehydration proceeds effecting changes in the dimensions of unit cells, e.g. cromolyn sodium. The other subcategory comprises

the planar hydrates, which are channel hydrates in which water is localized in a two-dimensional order, or plane, e.g., sodium ibuprofen. The third category (class 3) of crystalline hydrates are the ion-associated hydrates, in which the metal ions are coordinated with water, e.g., catteridol calcium [8,9].

In this section, some examples of nonstoichiometric hydrates and their characterization will be discussed in detail because these forms pose a special challenge in dosage form development due to unpredictability of water content in the crystals. Following the work of Cox et al. [70] the unusual water uptake and formation of nonstoichiometric hydrates of cromolyn sodium was reinvestigated using single crystal X-ray diffractometry, PXRD, as well as by molecular modeling [71]. Cromolyn sodium, an antiasthmatic drug, exists as two liquid crystalline phases and a crystalline hydrate phase that sorbs and liberates water continuously and reversibly to give a continuous range of nonstoichiometric hydrates [70]. The changes in the PXRD patterns of the crystalline hydrate phase of cromolyn sodium in response to the surrounding relative humidity (RH) were explained in the light of the molecular and crystal structure of cromolyn sodium. Single crystal X-ray diffractometry indicated the space group for cromolyn sodium as *P*1, a chiral space group, even though the molecule itself is achiral. The crystal structure of cromolyn sodium with five or six water molecules per cromolyn sodium molecule, solved at room temperature by Hamodrakas et al. [72], revealed the positions of only one sodium ion and two water molecules and showed that the second sodium ion and the other water molecules are disordered. Recently, the single crystal structure of cromolyn sodium at 76% RH, with 6.44 molecules of water was solved at 173 K by Chen et al. [71]. This work showed that the second undetermined sodium ion is disordered over three sites and that four of the eight water positions are partially occupied. Comparison of the crystal structures determined by Hamodrakas et al. [72] and Chen et al. [71] indicated that the cromolyn anion is flexible. In particular, the bond and torsional angles of the 2-hydroxypropane linking the two cyclic moieties, changed to accommodate lattice expansion or contraction resulting from water sorption and desorption by the crystals. As water is taken up, the relative occupancies of the sites of the

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second sodium ion and that of water molecules change. As a result, the triclinic structure with $\alpha > 90^\circ$ approaches the monoclinic form with $\alpha \approx 90^\circ$. To summarize, the presence of large water channels, the flexibility of the 2-hydroxypropane link, the disorder of the second sodium ion (Fig. 10) and the disorder of the surrounding water molecules in the crystal lattice explain the reversible and nonstoichiometric water sorption and desorption by cromolyn sodium. This study emphasizes the importance of the detailed single crystal structure in explaining many unusual physico-chemical properties of drug hydrates.

The muscarinic agonist, LY297802 tartarate [i.e., (+)-3-[3-(butylthio)-1,2,5-thiadiazol-4-yl]-1-azabicyclo[2.2.2]octane monohydrogentartrate}, was also found to exhibit an unusual tendency to form nonstoichiometric hydrates of variable, but specific composition, ranging from 0 to 0.5 mol of water [73]. Solid-state ^{13}C -NMR spectroscopy, in conjunction with moisture sorption analysis and X-ray crystallography was used to provide unique insights into nonstoichiometric moisture sorption behavior. The PXRD patterns of the drug exposed to different RH values (0 to 75%), indicated neither a peak shift nor the presence of any new peaks, suggesting that

the anhydrous and the hydrated forms of the drug are isomorphic. Fig. 11 shows the significant changes in the SSNMR peaks on exposure to different relative humidities and temperature, indicating that water incorporated into the crystal lattice changes the local chemical environment and causes the observed NMR changes. The incorporation of water into the crystal lattice of the drug was also confirmed by X-ray crystallography. The considerable hygroscopicity of the drug was rationalized in terms of the similar crystal structures of the hydrated and nonhydrated forms, and hence no significant structural modifications are needed for the reabsorption of water into the solids. The rates of dehydration and rehydration are largely determined by the size of the water channels and the strength of the hydrogen-bonding interactions that bind the water molecules in the channels.

Another interesting study with different solvated forms of L-lysine monohydrochloride (LH) was conducted by Bandyopadhyay et al. [74]. LH was found to form: a pure methanol solvate at water activity, $a_w < 0.34$, with methanol activity, $a_m > 0.7$; a dihydrate at $a_w > \sim 0.65$ with $a_m < 0.45$; and mixed solvates at intermediate values of a_w and a_m . It was found that the dihydrate and the mono-

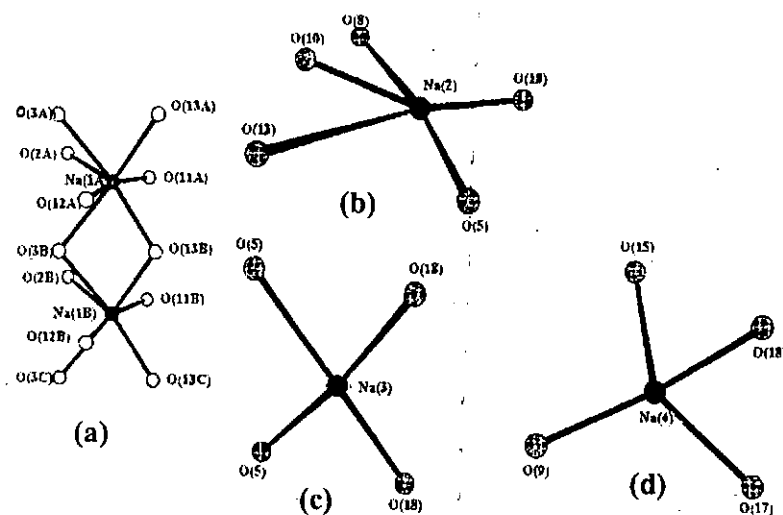


Fig. 10. In hydrated cromolyn sodium, coordination environment of: (a) the first (ordered) sodium ion, Na(1), shown in two neighboring unit cells (A and B); and the second (disordered) sodium ion at the three partially occupied sites, (b) Na(2), (c) Na(3), and (d) Na(4). The striped circles represent the sodium sites. The open circles represent the oxygen atoms coordinated to Na(1). The dotted (gray) circles represent the oxygen atoms coordinated to Na(2), Na(3), or Na(4) ([71], reproduced with the permission of the American Pharmaceutical Association).

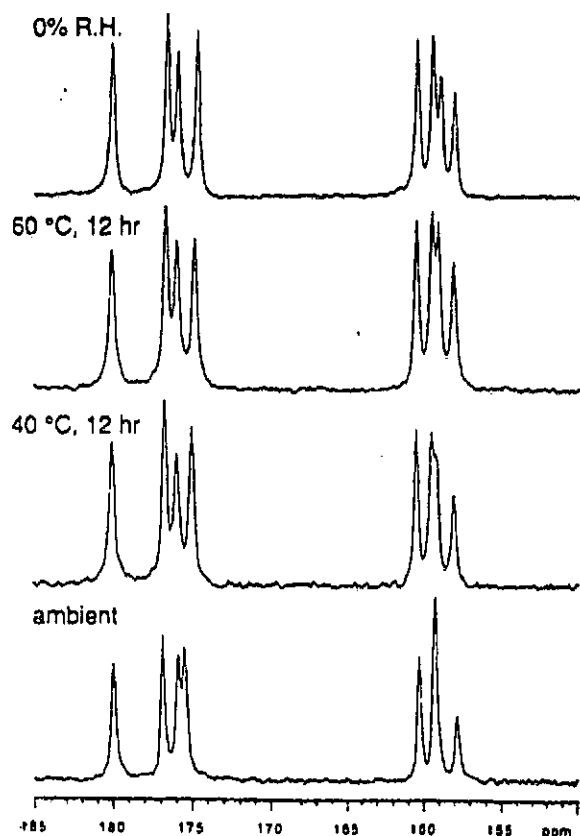


Fig. 11. Solid-state ^{13}C -NMR spectra of LY297802 tartrate ((+)-3-[3-(butylthio)-1,2,5-thiadiazol-4-yl]-1-azabicyclo[2.2.2]octane monohydrogentartrate) after storage at 0% humidity and before and after drying at elevated temperatures ([73], reproduced with the permission of the American Pharmaceutical Association).

methanol solvated forms of LH possessed similar crystal structures, similar PXRD patterns, but differed in the crystal habit. The crystal structures of LH hydrate and methanol solvate indicate that one molecule of methanol in the methanol solvate occupies approximately the same volume as the two water molecules in the dihydrate. During dehydration in the presence of methanol in the crystallization medium, the loss of one or more water molecules from the crystal lattice was compensated by the gradual uptake of methanol into the crystal structure to satisfy the hydrogen bonding pattern within the lattice with minor rearrangements, giving rise to mixed solvates. The similarities of the crystal lattices of the dihydrate and monomethanol solvate explain the similarity of the PXRD patterns.

3.3. Phase transformation of hydrates and solvates

Phase changes due to hydration/dehydration and solvation/desolvation of pharmaceutical compounds during processing or in the final product may result in an unstable system that would effect the bioavailability of drug from solid dosage forms. Various types of phase changes are possible in solid-state hydrated or solvated systems in response to changes in environmental conditions, such as relative humidity, temperature and pressure. For example, some hydrated compounds may convert to an amorphous phase upon dehydration and some may convert from a lower to a higher state of hydration yielding forms with lower solubility. Alternatively, a kinetically favored but thermodynamically unstable form may be converted during pharmaceutical processing to a more stable and less soluble form [8]. The phase transitions in hydrates and solvates can occur at various stages of dosage form development. Morris [9] has discussed the behavior of hydrates during processing, handling and storage of formulations in detail.

The phase transformations associated with exposure to water, such as during solubility measurements, wet granulation processes, dissolution studies and accelerated stability tests are likely to occur via solution mediation. Solution mediated phase transformations depend upon the solution phase to provide the mobility necessary to rearrange in the most stable form and hence are much faster than solid-state transformations. The rate of a solution-mediated transformation is proportional to the solubility of the species involved. Temperature, pressure and relative humidity may increase the rate of phase transformation of hydrates by inducing mobility in the system.

Solution-mediated phase transformations have been reported for many hydrate systems, such as theophylline crystals [17], eprosartan mesylate [75] and nedocromil sodium [76]. Ghosh and Grant [77] have addressed a common problem associated with the characterization of solvates which centers around the determination of solubilities of solvates and of nonsolvates that undergo phase transformation in the presence of an interacting solvent, such as solvation of nonsolvates in the solvent of crystallization or the desolvation of solvates in water. A thermodynamic cycle analogous to Hess's law but based on free

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energies has been developed to predict the theoretical solubilities of 1,2-dialkyl-3-hydroxy-4-pyridones, which form 1:1 formic acid solvates in the presence of formic acid, and of the 1:1 formic acid solvates which produce the corresponding unsolvated compounds in the presence of water. A good correlation was obtained between the solubility values measured by the standard extrapolation method and that calculated by means of the thermodynamic cycle.

Apart from identifying and characterizing the phases during various stages of drug development, it is very important to gain an understanding of the dehydration/hydration mechanisms and kinetics. Many models have been developed to account for the dehydration kinetics of the crystalline hydrates [78]. Nucleation is the most significant phenomenon in determining the transformation kinetics, that is, the rate of formation of a new phase [8]. The dehydration kinetics to some extent will also depend upon the class of the hydrate system to which the drug belongs, particle size and morphology. The practical applications of understanding the dehydration kinetics, as indicated by Morris [9], are mainly the determination of the conditions for allowable exposure of bulk drug substances during development and processing, proper packaging, allowable temperature ranges for shipping, storage, and labeling of the final product, and the initial selection of a form for development.

3.4. Prediction of the formation of hydrates and solvates

Predicting the formation of solvates or hydrates of a compound and the number of molecules of water or solvent incorporated into the crystal lattice of a compound is complex and difficult. Each solid compound responds uniquely to the possible formation of solvates or hydrates and hence generalizations cannot be made for a series of related compounds. Certain molecular shapes and features favor the formation of crystals without solvent; these compounds tend to be stabilized by efficient packing of molecules in the crystal lattice, whereas other crystal forms are more stable in the presence of water and/or solvents. There may be too many possibilities so that no computer programs are currently available

for predicting the crystal structures of hydrates and solvates.

3.5. Characterization of hydrates and solvates

The common methods for the characterization of hydrates and solvates are polarized light microscopy and hot stage microscopy, DSC, TGA, Karl Fischer titrimetry, single-crystal X-ray diffractometry, powder X-ray diffractometry, and infrared spectroscopy. These methods have been reviewed in detail [21] and will also be discussed in detail in later chapters.

Pressure DSC is gaining increasing popularity in the study of solvates and hydrates where dehydration reactions occur above or near the boiling point of water. Using conventional DSC, it is very difficult to measure the heats of dehydration and heat of vaporization separately, but if one conducts DSC experiments at elevated pressures, the two processes may be completely separated. The advantage of using pressure DSC is that the pressure can be precisely controlled and the solids can be subjected to a controlled temperature program while under substantially elevated temperatures. The influence of elevated pressures on the solid-state behavior of carbamazepine dihydrate was studied by Han and Suryanarayanan [79]. In Fig. 12 it is shown that pressure DSC can separate the dehydration and vaporization endotherms of carbamazepine dihydrate during its conversion to the anhydrate form. Also the technique permitted the water liberated on dehydration to remain in intimate contact with the anhydrous phase formed which could significantly influence its solid-state properties.

The combined physical analytical techniques of thermogravimetry and infrared spectroscopy (TG/IR) can permit identification of the solvent incorporated into the crystal lattice. This combined technique has been used to study formulated products, such as capsules and tablets [80].

4. Current challenges and future directions

4.1. Origins of the challenges

A series of flow charts and decision trees have been presented and discussed [11,22] that can be

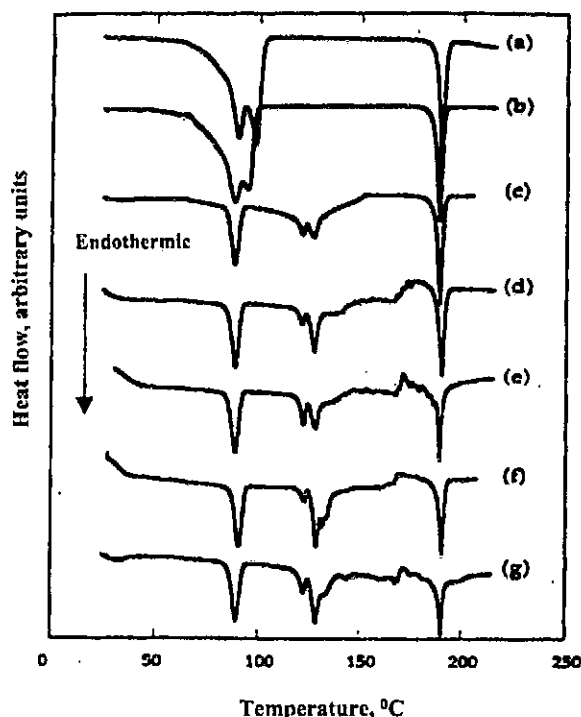


Fig. 12. Differential scanning calorimetry (DSC) curves of carbamazepine dihydrate at different pressures: (a) at atmospheric pressure in a conventional DSC cell, (b) at atmospheric pressure in a pressure DSC (PDSC) cell, (c) 100 p.s.i., (d) 200 p.s.i., (e) 300 p.s.i., (f) 400 p.s.i., and (g) 600 p.s.i. (1 p.s.i. = 6.9 kPa) ([79], reproduced with the permission of Elsevier Science).

used by investigators to characterize the polymorphs and solvates of compounds under development or for registration with regulatory authorities. Due to the complex and nonconventional behavior of various organic drug molecules, there are many opportunities for research and development in the area of characterization of polymorphs and solvates. Some of the problems which are commonly encountered during characterization of crystalline solids and which need to be addressed are: disorder in the crystal lattice due to pharmaceutical processing leading to conversion of a crystalline phase to an amorphous material or phase conversion from one form to the other; quantitating the amount of single polymorph in a mixture of polymorphs; identifying the solid form of the active ingredient in the formulated product, particularly when the drug is a minor component in the presence of numerous other materials (excipi-

ents); and the issues of disappearing polymorphs and the appearance of new polymorphs. In the following sections we will address some of these issues and some of the studies that have addressed these problems.

4.2. Phase transformations during processing

The effects of pharmaceutical processing on the crystalline state of drug polymorphs and solvates have been discussed recently by Brittain and Fiesco [16]. Exposure to changes in temperature, pressure, relative humidity and comminution are encountered during processes such as drying, granulation, milling and compression. The stresses applied to crystals during pharmaceutical processing can cause defects in their crystal lattices, and contribute to lattice disorder, thus affecting the physical properties of the resulting powder [81]. This problem has been discussed in detail by Byrn et al. [10]. Arising from different degrees of crystalline disorder, the difficulty in reproducing materials with the same properties is a major concern in the pharmaceutical industry.

Milling, the last processing step in the production of bulk drug substance to reduce particle size, is often accompanied by a decrease in crystallinity due to the creation of lattice defects, beginning at the surface. The defects created by mechanical activation of the solid on the surface can migrate, transform, and change their number and nature. If the defects in the mechanically activated crystal heal to produce a crystal lattice different from the initial lattice, then a polymorphic transformation has taken place. Milling-induced polymorphic changes have been observed for many small drug molecules, such as fostedil, chloramphenicol palmitate, indomethacin and phenylbutazone [16]. Polymorphic transformation of the dipeptide sweetener, aspartame hemihydrate, can occur during milling [82]. Polymorph II of aspartame hemihydrate was found to transform to form I during ball-milling or on heating for 30 min at 160°C in the presence of steam as shown in the X-ray diffraction pattern (Fig. 13). The susceptibility of form II of aspartame hemihydrate to transform to form I has been attributed to the less symmetric crystal structure of form II compared to that of form I as studied by spectroscopic methods.

Some authors, such as Hüttenrauch [81] have

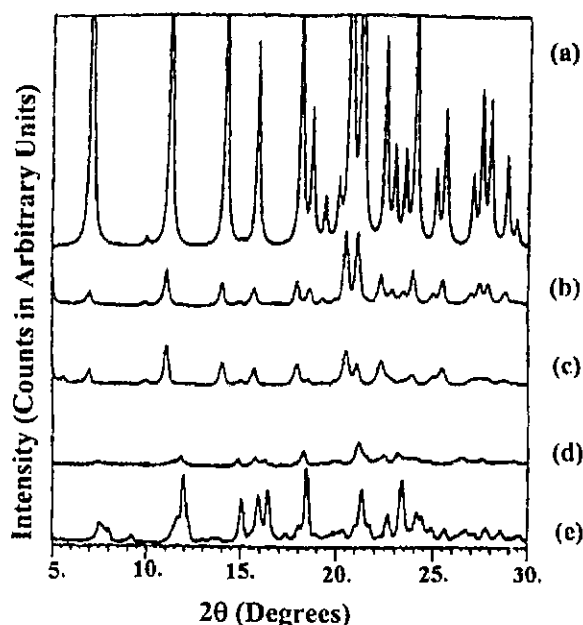


Fig. 13. Powder X-ray diffraction patterns of aspartame hemihydrate: (a) theoretical powder pattern calculated from the crystal structure of aspartame hemihydrate (form I) determined by Hatada et al., (1985) [99], (b) experimental powder pattern of the ball-milled aspartame hemihydrate (form I), (c) experimental powder pattern of aspartame hemihydrate that had been heated for 30 min at 160°C in the presence of steam (form I), (d) experimental powder pattern of aspartame hemihydrate after compression at 250 MPa for 1 min (form II), and (e) aspartame hemihydrate as received (form II) ([82], reproduced with the permission of the American Pharmaceutical Association).

suggested that the trauma to crystals during grinding may lead to a decrease in crystallinity, which should improve the compression capacity and dissolution rate of the drug molecules. This hypothesis was tested by studying the morphology, crystalline state, compression capacity and dissolution properties of native and ground crystals of aspirin and lactose monohydrate [83]. No significant increase in compression capacity was observed when native and ground crystals were compared. Only a slight increase in the dissolution rate was observed for ground aspirin crystals which was attributed to surface defects due to grinding resulting in improved crystal wetting.

The effect of roller compaction on lattice defects and phase change has been examined for aspirin [84]. The water vapor sorption isotherm obtained for aspirin on roller compaction indicated much more

water uptake than had been reported previously for other crystalline samples of aspirin under similar conditions. Various possibilities were suggested for the unusual water uptake of aspirin on roller compaction, such as formation of a polymorphic form of aspirin with a much greater affinity for water, formation of a crystalline hydrate in the aspirin sample, or significant reduction in particle size of the aspirin particles thereby providing an increased specific surface area for water vapor adsorption. It is also possible that roller compaction disrupted the crystalline order of some part of the aspirin crystals forming amorphous regions, which then take up relatively large quantities of water into their bulk structure. This example clearly indicates that processes, such as roller compaction, can introduce considerable disorder in the surface of crystals leading to a marked increase in the tendency to sorb water vapor.

Thermal activation, like mechanical activation during processing, also results in a high-energy state of crystals that may reorganize into a different lattice arrangement resulting in a phase change. The thermal stability of drug substances is important, because formulations are often dried at elevated temperatures after wet granulations so that the tablets may contain small regions of high temperature (hot spots) during compression. Various examples have shown that a change of temperature may influence the stability of drug molecules [16]. The effect of low temperatures, such as during freeze-drying, on the crystalline form of the drug has also been studied. The formation of a new mannitol hydrate during freeze-drying has been reported [85]. The formation of a crystalline hydrate by an excipient during freeze-drying may have several practical consequences, such that the difficulty of removing bound water from the crystal lattice can significantly limit the drying rate, while the residual water that is not removed by freeze-drying may be a potential threat to product stability if it is released during storage. The mannitol hydrate formed during freeze-drying survived the typical drying cycle and converted to the anhydrous polymorph of mannitol upon heating.

Spray drying has also been shown to lead to loss of crystallinity in materials, by a combination of processes involving rapid solidification of dissolved material and solid-state transitions due to milling effects in the atomiser. Spray drying leads to conver-

sion of a crystalline phase to an amorphous state and, because the amorphous state is metastable with respect to the crystalline form, phase transformations are likely to occur within the shelf life of the pharmaceutical product, resulting in loss of quality and potency in the product [86].

In view of the significant effects that the state of disorder in crystalline solids caused by pharmaceutical processing can have on the properties of pharmaceutical solids, it is important to be able to assess the extent of disorder in a solid quantitatively down to very low levels. Various methods have been used to measure the percent disorder, such as using predetermined mixtures, measurements of X-ray powder diffraction, density and heats of crystallization which revealed limits of detectability down to about 10%. Using water vapor sorption measurements under very carefully controlled conditions, it was possible to detect disorder as low as 1% in milled samples of sucrose [87]. A comparison of the four methods mentioned above for estimating the percent disorder of milled samples of sucrose gave very consistent results, once the underlying factors that make these techniques sensitive to the concentration of amorphous content were recognized and taken into account.

4.3. Degree of crystallinity

The previous section has emphasized that many pharmaceutical processes lead to a decrease in crystallinity of drug phases. Various studies have concluded that the formation of amorphous material during processing is highly undesirable. The amorphous material, being in a thermodynamically metastable state, is susceptible to reconversion to the crystalline state, affecting many physico-chemical characteristics of the drug. A later chapter provides detailed coverage of amorphous materials. An estimation of the degree of crystallinity of a sample before and after processing poses one of the larger challenges facing the pharmaceutical field. Powder X-ray diffractometry is still the commonly used method for determining the degree of crystallinity, though this method suffers from some limitations due to peak broadening, amorphous halo, and preferred orientation which make interpretation and quantitation difficult. DSC may not be a sensitive method for measuring crystallinity due to crystalliza-

tion of the amorphous content at elevated temperatures and the effects of differences in heat capacity. Solution calorimetry has been proposed as an accurate method for analysis of percent crystallinity [11,88,89]. A decrease in the endothermic enthalpy of solution indicates a decrease in the crystallinity of the sample. However, differences in surface area produced by grinding or by other processing techniques can also result in changes in the heat of wetting of a sample. Judicious choice of solvent can be employed to reduce such surface effects, which themselves contribute to the observed crystallinity of the sample.

Near infrared (NIR) spectroscopy is another technique being used to measure the degree of crystallinity, and has also proved useful in studies of the polymorphism and water content of sugars. The NIR spectrum of a sample contains both physical and chemical information. Being noninvasive, nondestructive and operable at room temperature, the method is a valuable tool with which to assess changes in the amorphous and crystalline state of lactose [90]. NIR has been used to follow the changes in the amorphous state, the onset of crystallization, and the changes between α - and β -lactose, which accompany the onset of crystallization. In another study, the nucleation and crystallization kinetics of amorphous lactose was investigated by gravimetry in an automated vacuum moisture balance. The combination of isothermal and nonisothermal activation energies allowed the investigation of both crystal growth and nucleation mechanisms and led to the separation of activation energies for nucleation and growth [91].

4.4. Characterization of mixtures of polymorphs

Another common problem encountered during drug development is quantitative control of the proportion of polymorphic forms present in a mixture. According to the US FDA regulations, the method of analysis for the proportion of forms must be validated, and also the proportion of forms must remain within stated limits through the retest date of the drug substance and potentially throughout the shelf life of the product. This is a very onerous requirement, especially if the forms have a tendency to interconvert. Byrn et al. [11] suggested that the best way to deal with this problem is probably by

developing methods to prepare only one crystal form and maintaining this form throughout processing. Powder X-ray diffractometry is often a useful method to determine the percentages of polymorphs in a mixture. However, the detection limit is variable from case to case, and is sometimes as high as 15%. It is therefore important to develop sensitive analytical methods with a lower limit of detection.

Attenuated total reflectance (ATR) FTIR spectroscopy has been shown to be valuable for the quantitative analysis of the polymorphic content of bulk pharmaceutical materials. The feasibility of using ATR-FTIR for the qualitative and quantitative analysis of mixtures of pharmaceutical polymorphs has been studied using three known polymorphs of ganciclovir as a model compound [92]. Definitive identification and quantitation of all three polymorphs could be achieved using ATR-FTIR spectroscopy in conjunction with partial least-squares modeling. This technique has many advantages, such as speed, nondestructiveness, relative ease of use, and most important, no sample pretreatment before measurement.

Raman spectroscopy is another technique that is being widely used to quantitatively estimate the percentage of one polymorph in a mixture of polymorphs. FT-Raman spectrometry offers many advantages, the most prominent being, minimal sample preparation, sensitivity to polymorphism, and noninterference from water. Two polymorphs of fluconazole were characterized using FT-Raman spectroscopy and principal components regression using cross validation provided quantitative analysis of the percentage of one polymorphic form in the mixture of other forms [93]. A novel sample holder was developed whereby the sample is held in an NMR tube which is rotated around its axis and at the same time moved up and down. This method of sample presentation leads to a large increase in the volume studied and is important for inhomogeneous samples for which sub-sampling is a problem. Possible degradation of the sample through heating by the laser can also be avoided [94].

X-Ray powder diffractometry is still the common method for the quantitative estimation of polymorphs in a mixture of polymorphs. This method requires that at least one high-intensity peak unique to each form is available for intensity measurements and that

the plot of the peak intensity ratio as a function of the weight ratio of the components should result in a straight line. Modern computer controlled X-ray powder diffractometers now permit quantitative analysis of multicomponent mixtures using the complete powder diffraction profile rather than a limited amount of low-angle integrated intensity data. Artificial neural networks (ANNs) in quantitative X-ray powder diffractometry were used successfully to identify and quantify the two known modifications of ranitidine hydrochloride even when the weight fraction of one polymorph in the mixture was as low as 0.01 [95]. ANNs have been used mostly in problems of pattern recognition and modeling, and is therefore useful in deciphering the pattern in diffraction data from polymorphic mixtures. The ANNs model predicted concentration precisely, accurately, and with minimal bias through a wide range of ratios of the two known ranitidine hydrochloride polymorphic forms in a mixture (Fig. 14). This method minimizes the problems associated with preferred orientation and overlapping X-ray lines. The same group of researchers has shown the potential of ANNs in combination with DRIFT spectroscopy to analyze the polymorphic purity of crystalline ranitidine hydrochloride as a bulk drug and as an active ingredient of

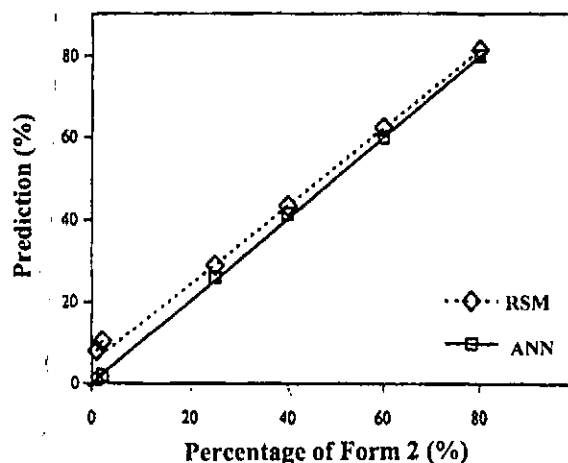


Fig. 14. Predicted concentrations of the two polymorphic forms (form 1 and 2) of ranitidine hydrochloride by the response surface methodology (RSM), a statistical modeling method, and by the artificial neural networks (ANNs) method, plotted against measured percentage of form 2 ([95], reproduced with the permission of Elsevier Science).

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a tablet formulation. Simultaneous identification and quantification of all the ingredients in the tablet formulation was possible. This study has shown that the complex problem of quantifying a drug in mixtures containing two or more components with overlapping spectra can be solved by the DRIFT-ANNs technique [96].

Another technique, which is gaining popularity in the quantitative analysis of mixtures of polymorphs, is solid-state ^{13}C -CP-MAS NMR spectroscopy. This method was preferred for quantitative analysis of polymorphic mixtures of the herbicide, pendimethalin, which exists as two polymorphs with different colors and crystal habits [27]. ^{13}C -NMR provided the most sensitive and definitive evidence of the transition from the yellow to the orange form. This method enabled as little as 2% of orange pendimethalin to be determined in a sample consisting mostly of the yellow polymorph. It is the least invasive of the instrumental methods and can be used to detect the ratio of the two polymorphs in solid formulations.

A related challenge faced by the pharmaceutical industry is the determination of the polymorphic nature of the drug in the presence of excipients in a dosage form especially when the active drug is present as a low percentage of the overall formulation [97]. This problem can be addressed by developing sensitive techniques with lower limits of detection or by using a combination of techniques.

5. Conclusions

In order to save time and cost it is very important to choose the most suitable form of the crystalline drug in the initial stages of drug development. In recent years a good deal of research has been directed towards achieving this goal. Systematic isolation and early characterization of the largest number of possible forms of a drug reduces the chances of surprises at the late production stage due to identification of a new crystalline form or phase change. With the development of more sophisticated computational tools, the main focus of many investigators is to be able to predict all the possible forms of a drug from its molecular structure. Understanding the origins of the multiple solid forms of a drug

molecule, either due to differences in packing arrangement or conformation of the molecules, becomes the first step in prediction. Single crystal X-ray diffractometry and solid-state NMR spectroscopy are two techniques that are gaining increased application in determining the various crystal structures and the origins of polymorphism and pseudopolymorphism of a particular drug. When crystal structures can be calculated with certainty, it will be possible to predict the various polymorphs of a compound and this information could be used to guide experimental studies. This goal may be difficult to achieve owing to the complex molecular structures of new organic molecules and the presence of several molecules in each asymmetric unit, but the future development of improved force fields and increased computational speeds, may make it achievable.

Improved experimental methods leading to more accurate and detailed phase diagrams are also finding increased use in determining the stability of various polymorphs. It is important to make every effort to prepare and to identify the most stable polymorph in order to guide the selection of the optimal form for development. The emergence of sensitive methods and the use of combination techniques, facilitate the identification and the more accurate characterization of the various polymorphs of a drug molecule. One of the main analytical challenges faced by the pharmaceutical analyst is the development of better quantitative methods for identifying a single polymorph in a mixture of polymorphs and for determining the percentages of amorphous or crystalline content of the drug. More and more sensitive methods are being developed to address this problem.

An increased understanding of the phenomenon of polymorphism should enable pharmaceutical scientists to gain control over the crystallization process in order to selectively obtain the desired polymorph or suppress the growth of an undesired one. Phase changes during processing and scale-up are a problem, which may be avoided by carefully designed initial small-scale studies. The availability of detailed structural data, combined with strategic design of substrates and additives, has led to significant advances in the control over the polymorphs obtained in a particular crystallization [98]. With all the information available from these initial studies, it

should be possible to design and to select processing conditions which would give a desired polymorph and maintain the desired form throughout the various stages of drug processing and manufacture.

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